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VITAMIN A IN BEEF, PORK, AND LAMB[1]

By RALPH HOAGLAND, *Senior Biochemist*, and GEORGE G. SNIDER, *Chemical Laboratorian, Biochemic Division, Bureau of Animal Industry, United States Department of Agriculture*

INTRODUCTION

Until comparatively recently the term "vitamin A" has been used synonymously with "fat-soluble A" to denote that vitamin which is found most abundantly in cod-liver oil, egg yolk, butter fat, liver, and certain other products. It is now believed, however (*2*, *5*, *6*, *7*),[2] that fat-soluble A really consists of two vitamins. One of these is necessary for growth in young animals, its absence being indicated by nutritive failure, by the development of ophthalmia, and by certain other symptoms. The other is essential for the normal development of bones, and its absence is denoted by a disturbed calcium and phosphorus metabolism resulting in the onset of rickets. The first of these fat-soluble vitamins is now designated as vitamin A, while the other is commonly known as the antirachitic vitamin. It is with the first of these vitamins, vitamin A, that this paper is concerned.

Adequate information concerning the distribution of vitamin A among our foodstuffs is much to be desired in order that such foods as will provide an abundance of this vitamin may be selected for the diet. Since meat is one of our most important food products, it is desirable to have more information regarding its value as a source of vitamin A than is now available. It is important, also, to know the extent to which vitamin A is stored in the muscles of the ox, the sheep, and the hog, because of the bearing that such reserves have upon the vitamin A requirements of those animals.

Although meats are generally considered to be very deficient in vitamin A, it appears that relatively little systematic work has been done on the subject. McCollum, Simmonds, and Parsons (*4*) found that rats made very poor growth when fed rations containing even very large proportions of dried lean beef as the source of vitamin A. They conclude that "muscle tissue is very deficient in these vitamins but does not entirely lack any one of them."

Wright (*8*) determined the vitamin A content of pork that had been held in cold storage for nine years, the feeding tests being carried on with young chickens. The amount of pork fed to each group of chickens is not stated, but the birds that were fed the rations containing pork as the source of vitamin A made practically as good growth as those fed a normal ration.

The purpose of this investigation was to determine the vitamin A content of beef, pork, and lamb as measured by the growth induced in young albino rats when fed a ration adequate in other respects but containing dried lean meat as the sole source of vitamin A.

[1] Received for publication August 28, 1924; issued September, 1925. The authors extend their thanks to Oliver P. Clipper, assistant laboratorian, for assistance rendered in conducting the feeding tests.

[2] Reference is made by number (italic) to "Literature cited," p. 221.

EXPERIMENTAL WORK

Description and Care of Breeding Rats

The albino rat was used in all the tests reported in this paper. The foundation stock for the breeding colony was obtained from E. C. Schroeder, in charge of the Experiment Station of the Bureau of Animal Industry, United States Department of Agriculture, at Bethesda, Md. A continual process of selection and elimination has been practiced in breeding the rats, and the colony is healthy, vigorous, and prolific. The average weight of mature males is approximately 300 grams and of females 190 grams. The number of young in a litter ranges from 4 to 16, the average being approximately 8. The rats, both breeding and experimental, are kept in a well-lighted room with a southern exposure; but, on account of certain obstructions in front of the windows, only a very few of the rats are exposed to the direct rays of the sun.

The following-described ration is at present being fed to the breeding rats, and it has not been modified materially for some time:

Ration for breeding rats

	Per cent		Per cent
Yellow corn	27. 5	Dried yeast	4. 0
Oatmeal	20. 0	Calcium carbonate	4. 0
Wheat	25. 0	Sodium chloride	0. 5
Dried beef	15. 0		
Dried egg or egg yolk	4. 0		100. 0

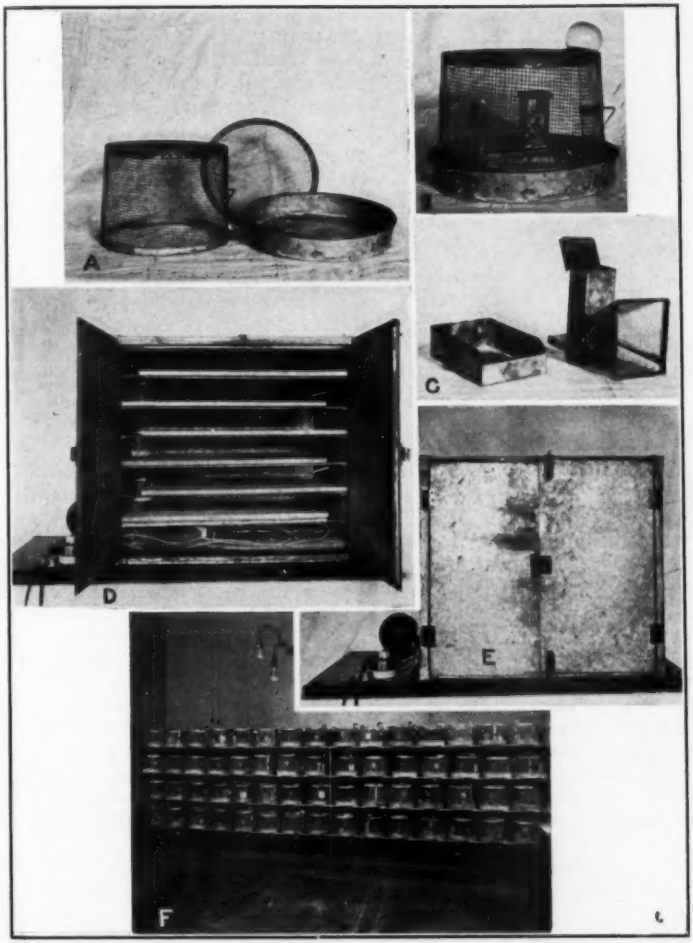
In addition to this ration the rats are given fine-cut cabbage at frequent intervals and water.

System of Testing for Vitamin A

Young rats weighing approximately 40 grams each and not exceeding 30 days in age, when they reach that weight are selected for the tests. As a rule, four rats are fed each ration. No attempt is made to select rats from different litters, and the sexes are ordinarily about equally divided. Each rat is weighed at the beginning of the test and at regular semiweekly intervals thereafter for a period of 90 days, unless the experiment is terminated earlier by nutritive failure or by some untoward circumstance.

Each rat is kept in an individual, cylindrical-shaped cage with a separate pan bottom, as shown in Plate 1, A and B. The dimensions of the cage are as follows: The upper part is 10¼ inches in diameter at the top, 11¼ inches at the bottom, and 8 inches high. The heavy clip projecting midway on the outside prevents the cages sticking together when stacked. A hole in the top allows insertion of the glass drinking vessel, which is held in place by a removable spring clip on the inside of the cage. The pan bottom is 11¾ inches in diameter at the bottom, 12½ inches at the top, and 2¼ inches deep. The slight flare to the sides permits stacking. A circular screen made of ¼-inch mesh galvanized wire, and slightly smaller than the pan, is used to protect a circular sheet of heavy blotting paper which serves as an absorbent.

Each cage is provided with a self-feeder (*3*) as shown in Plate 1, C, which holds approximately 100 grams of feed. By this means an accurate record is kept of the feed consumed.



A.—Cage used for housing individual experimental rats, showing parts

B.—Rat cage set up ready for use. The self-feeder and drinking vessel have been placed in the cage

C.—Self-feeder for rats showing parts

D.—Drying oven with doors open showing pans for holding meat or casein and electro-heating units on the bottom of the oven

E.—Oven used for drying meat and for drying casein. The inside dimensions are 30 by 27 by 20 inches. The walls and doors are 1 inch thick and are filled with asbestos. The oven is made of galvanized iron. The blower shown at the left has a 2½-inch outlet and is run by a one-tenth horsepower motor. The air outlet is at the upper right end of the oven and lower down are shown thermometers. The switch in front of the blower controls the heating units in the bottom of the oven

F.—View in animal laboratory of the biochemic division showing vitamin feeding tests in progress. This stack of shelves accommodates 128 cages and a like number of rats

As a routine procedure each rat on test is cared for daily, except Sunday, as follows: The self-feeder is examined and any feed that has been pulled into the catch pan or base is returned to the hopper, as well as any feed that has been scattered on the bottom of the cage. When nearly empty the feeder is removed and weighed, and it is replaced by a clean feeder filled with feed. The blotting paper is changed daily, except Sunday, and the drinking vessels are filled as often as necessary. The cages, feeders, and drinking vessels are cleaned and sterilized once a week.

A complete record for each rat is kept. Growth curves for the several rats that are fed the same ration are platted on one sheet. All rations tested are numbered serially.

Unless otherwise noted, the rations fed in the vitamin A tests were made up according to the following standard:

Standard ration

	Per cent
Protein (N x 6.25)	20
Fat	10
Ash mixture	4
Dried yeast	10
Starch, etc	56
	100

The 20 per cent of protein comprises that present in the dried meat which is being tested for vitamin A plus sufficient protein from dried vitamin-A-free muscle tissue or casein to make up the difference, the yeast protein not being included. The 10 per cent of fat is the sum of the fat in the dried meat and sufficient hardened cottonseed oil to make the required amount. The ash mixture is made up according to a formula of Drummond and Watson (*1*, *p. 237*). Dried baker's yeast and cassava starch comprise the balance of the ration. The proportion of protein in the ration, as well as that of dried yeast, is considerably higher than is necessary for normal growth; but this is a distinct advantage, since it guards against a deficiency of either protein or vitamin B becoming a limiting factor in the growth of the rats in case of reduced food intake due to a deficiency of vitamin A. In some rations in which relatively large proportions of dried meat were incorporated the percentage of protein, as well as that of fat, frequently exceeded the proportion stated in the standard ration.

Ash mixture

	Grams		Grams
Sodium chloride	5.2	Calcium superphosphate	16.2
Magnesium sulphate	8.0	Calcium lactate	39.0
Sodium dihydrogen phosphate	10.4	Ferric citrate	3.5
Dipotassium hydrogen phosphate	28.6	Potassium iodide	Trace.
		Manganese sulphate	Trace.

Purification of Ingredients in Ration

The greatest difficulty encountered in the estimation of the vitamin A content of a food product is the preparation of a basal ration free from vitamin A or containing a very small amount of it. After considerable experimentation the desired result has been accomplished. It was found that the cassava starch, dried yeast, and hardened cottonseed oil contained no vitamin A and so did not need purification.

In the earlier work reported in this paper, both the starch and the yeast were thoroughly extracted with ether before being used, but this practice was discontinued as soon as it was found to be unnecessary. Both the crude casein and the dried ox muscle used as sources of protein were found to contain considerable vitamin A and purification was required. Purified ox muscle was used in most of the tests reported in this paper, but later it was found more satisfactory to purify casein, and that product was then used exclusively as the supplementary source of protein in these vitamin A studies.

The ox muscle was purified as follows: Water-insoluble muscle tissue, a by-product resulting from the preparation of beef broth for the growth of tubercle bacilli, was dried, ground fine, and extracted with ether in large percolators until the material was practically free from fat. The extracted muscle was mixed with 60 per cent alcohol by weight in a large dish, transferred to large percolators, and extracted with 60 per cent alcohol until the extract was of a very light straw color. It was found to be practically impossible to obtain a colorless extract. The extraction with ether required two or three days and that with alcohol four or five days. The extracted material was dried and tested for vitamin A, but was found still to contain an appreciable amount of the vitamin. In order to reduce the vitamin content still further, the extracted muscle tissue was then heated in a current of air at an average temperature of approximately 115° C. for 24 hours, when it was found to be practically free from vitamin A. This method was found to be very tedious and later was abandoned.

The following method has been used regularly since October, 1923, in the purification of commercial casein, with very satisfactory results. Six hundred grams of granular casein is spread out in a thin layer in a shallow, galvanized-iron pan 18½ by 25½ inches by ½ inch deep, and five of these pans are set at one time in the drying oven shown in Plate 1, D and E. The casein is then heated in a current of air at an average temperature of 115° C. for 24 hours. The pans are then taken out and the casein is transferred to a large dish, thoroughly mixed, and returned to the pans. Heating is continued for another 24 hours, when the casein is nearly always found to be free from vitamin A as determined by feeding tests with rats. If the rats make more growth than is considered normal for the rats on a vitamin-A-free ration, the casein is heated for an additional 24 hours and tested again. Ordinarily about 25 pounds of casein is purified, thoroughly mixed, and tested in advance of requirements for the product. The heating changes the color of the casein to a very light brown but does not char the product. Comparative feeding tests have shown that rats fed a ration containing 20 per cent of protein from the purified casein made equally as good growth as those that received a ration containing a like percentage of the untreated casein, the rations being adequate in other respects.

Feeding Tests with Basal Rations

Five lots of purified ox muscle and one lot of purified casein, which comprise all of these products that were used as sources of protein in the feeding tests reported in this paper, were tested to determine their freedom from vitamin A, with the results shown in

Figure 1. The first two groups of rats, Nos. 204 and 222, respectively, made slightly more growth than the three other groups, and this is probably due to the presence of a small amount of vitamin A in the two lots of purified ox muscle fed the first two groups of rats. The growth made by the rats in the last three groups, Nos. 234, 297, and 343, represents about as good results as the writers have been able to obtain after having tested a considerable number more of highly purified basal rations. This indicates that the slight growth made by the

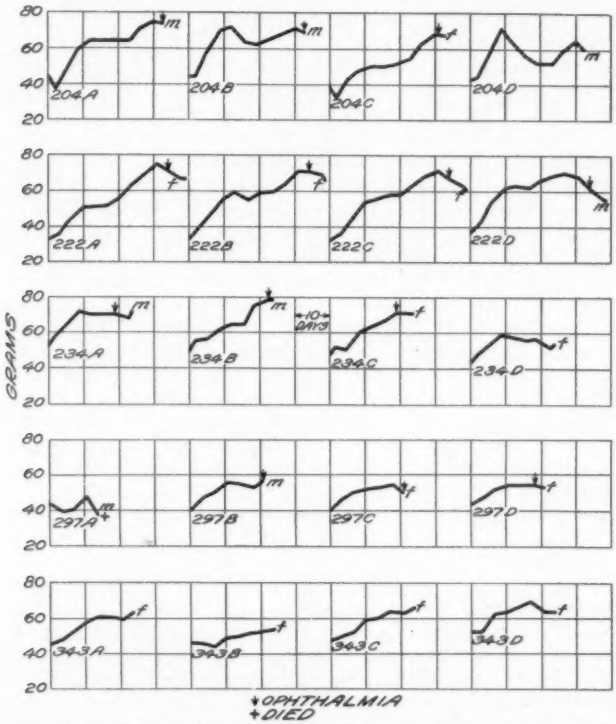


FIG. 1.—Basal rations. Graphs showing rate of growth of five groups of rats, each group being fed a basal ration practically free from vitamin A but otherwise adequate. The rations fed to the first four groups of rats, including animals Nos. 204 A to 297 D, were made up in parts by weight as follows: Ox muscle treated to remove vitamin A, to make 20 parts protein; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil sufficient to make 10 parts fat; and cassava starch to make 100. A different lot of ox muscle was used in each of the rations

The ration fed to rats 343 A to 343 D, inclusive, was made up in the same proportions, except that casein, treated to destroy vitamin A, was substituted for ox muscle

The sex of each rat is indicated by the letter at the end of each graph, *m* denoting male, *f*, female

last three groups of rats shown in Figure 1 was probably due to the reserve store of vitamin A in the tissues of the young rats, and that the growth made by the first two groups of rats was due chiefly to the same factor.

Ophthalmia developed in most of the rats, as indicated, but it is not the writers' practice to continue a feeding test for any considerable time after a rat ceases to gain in weight even though ophthalmia is not evident.

VITAMIN A IN BEEF

Description of Samples

The beef, which consisted of the rounds from the carcasses of fat steers, was purchased from local packers who slaughter their own cattle and from the branch houses of western packers. Two of the steers were slaughtered in Chicago, one in St. Louis, one in Omaha, and six in Washington, D. C. The rounds were purchased one at a time, as needed, and were prepared for use in the feeding tests in the following manner.

A cross section weighing from 20 to 25 pounds was cut from the thickest part of the round, the muscle tissue was trimmed free from fat and connective tissue, ground, mixed with water and toluol in the proportion of 800 grams of meat, 400 c. c. water and 40 c. c. toluol. The mixture was spread out in a thin layer in one of the shallow pans belonging to the drying oven shown in Plate 1, D and E. The oven was filled with five pans of the meat prepared in this manner, and the material was dried in a current of air at a temperature that did not exceed 60° C. The thin layer of meat became dry on the surface in 2 or 3 hours, when it was turned over by means of a spatula in order to expose the moist undersurface, and drying was continued overnight. The meat was thoroughly dried in from 20 to 24 hours,

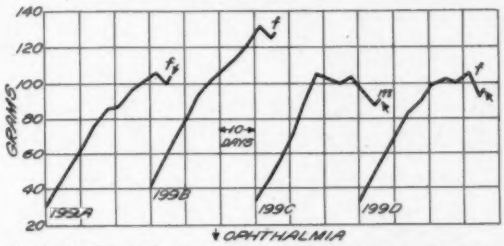


FIG. 2.—Vitamin A in beef. Graphs showing rate of growth of rats that were fed a ration containing 30 per cent of dried round steak as the source of vitamin A in an otherwise adequate diet. The ration was made up in parts by weight as follows: Dried round steak No. 869, 30; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 4.9; cassava starch, 51.1; total, 100

when it was ground fine and stored in stoppered bottles in the dark at a temperature of 34° to 36° F. until needed, the feeding tests being begun as promptly as possible. Each lot of dried beef was analyzed for nitrogen and fat before being used in a ration.

Feeding Tests with Beef

In Figure 2 are shown the growth curves of four rats that were fed a ration containing 30 per cent of dried beef No. 869 as the sole source of vitamin A in an otherwise adequate diet. The rats grew at a fair rate for a time, but growth soon ceased; 3 of the rats developed ophthalmia, and one of these had rhinitis also. It is evident that the amount of vitamin A supplied by the 30 per cent of dried beef in this ration was not nearly sufficient to meet the normal requirements of the rats.

In Figure 3 are shown the growth curves of four rats that were fed a ration containing 30 per cent of dried beef No. 874 as the sole source of vitamin A. These rats made less satisfactory growth than those shown in Figure 2. Three of the animals developed ophthalmia and two died. The amount of vitamin A supplied in this ration was far from adequate for the needs of the rats.

In Figure 4 are shown the growth curves of two groups of rats that were fed rations containing 15 and 30 per cent, respectively, of dried beef No. 902 as the sole source of vitamin A. Neither group of rats made much growth, about what might be expected from the reserve of vitamin A in their tissues, and every rat developed ophthalmia.

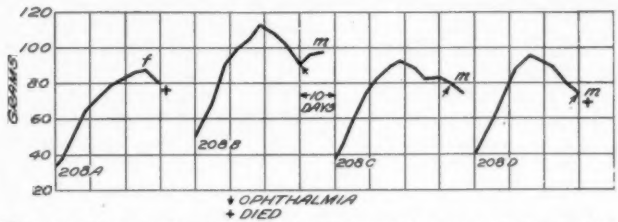


FIG. 3.—Vitamin A in beef. Graphs showing rate of growth of rats that were fed a ration containing 30 per cent of dried round steak as the source of vitamin A in an otherwise adequate diet. The ration was made up in parts by weight as follows: Dried round steak No. 874, 30; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 5.9; cassava starch, 50.1; total, 100

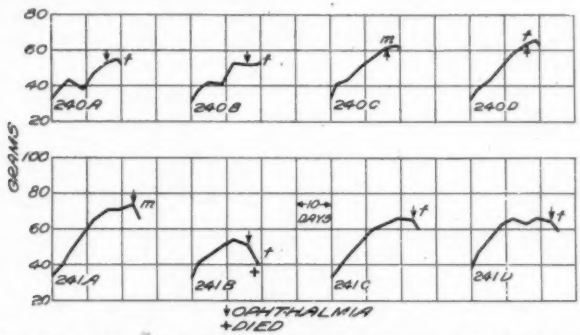


FIG. 4.—Vitamin A in beef. Graphs showing rate of growth of two groups of rats that were fed rations containing 15 and 30 per cent, respectively, of dried round steak as the source of vitamin A in otherwise adequate diets

The ration fed to rats Nos. 240 A to 240 D inclusive, was made up in parts by weight as follows: Dried beef No. 902, 15; purified ox muscle, 8.8; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 7.4; cassava starch, 54.8; total 100

The ration fed to rats Nos. 241 A to 241 D, inclusive, was made up as follows: Dried beef No. 902, 30; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 4.8; cassava starch, 51.2; total, 100

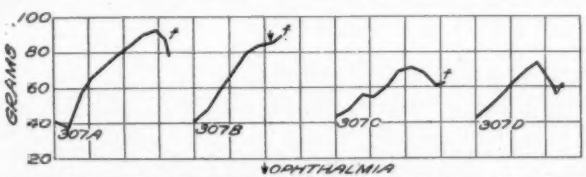


FIG. 5.—Vitamin A in beef. Graphs showing rate of growth of rats that were fed a ration containing 30 per cent of dried round steak as the source of vitamin A in an otherwise adequate diet. The ration was made up in parts by weight as follows: Dried beef No. 906, 30; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 3.2; cassava starch, 52.8; total, 100

In Figure 5 are shown the growth curves of rats that were fed a ration containing 30 per cent of dried beef No. 906 as the sole source of vitamin A. Two of the rats made appreciable growth, but the other two grew about as much as rats fed one of the more satisfactory basal rations. The difference in the growth of the two pairs of rats may be due to the fact that they were from different litters.

The growth curves of two groups of rats that were fed rations containing 15 and 30 per cent, respectively, of dried beef No. 912 as the sole source of vitamin A are shown in Figure 6. The rats getting the ration containing 30 per cent of dried beef made slightly more growth than the other group, but the amount of vitamin A in this ration was far from adequate, and every rat in the two groups developed ophthalmia.

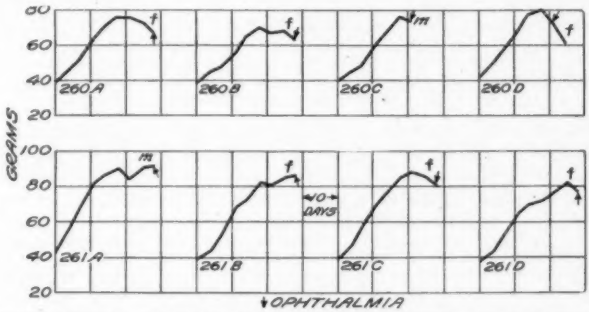


FIG. 6.—Vitamin A in beef. Graphs showing rate of growth of two groups of rats that were fed rations containing 15 and 30 per cent, respectively, of dried round steak as the source of vitamin A in otherwise adequate diets

The ration fed to rats Nos. 260 A to 260 D, inclusive, was made up in parts by weight as follows: Dried beef No. 912, 15; purified ox muscle, 9.2; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 7.6; cassava starch, 54.2; total, 100

The ration fed to rats Nos. 261 A to 261 D, inclusive, was made up as follows: Dried beef No. 912, 30; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 5.1; cassava starch, 50.9; total, 100

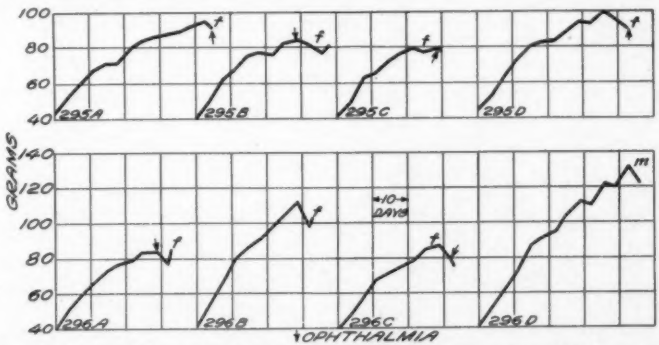


FIG. 7.—Vitamin A in beef. Graphs showing rate of growth of two groups of rats that were fed rations containing 15 and 30 per cent, respectively, of dried round steak as the source of vitamin A in otherwise adequate diets

The ration fed to rats Nos. 295 A to 295 D, inclusive, was made up in parts by weight as follows: Beef No. 934, 15; purified ox muscle, 9.4; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 7.2; cassava starch, 54.4; total, 100

The ration fed to rats Nos. 296 A to 296 D, inclusive, was made up as follows: Beef No. 934, 30; dried baker's yeast, 10; hardened cottonseed oil, 5.6; ash mixture, 4; cassava starch, 50.4; total, 100

In Figure 7 are shown the growth curves of two groups of rats that were fed rations containing 15 and 30 per cent, respectively, of dried beef No. 934 as the source of vitamin A. The average rate of growth of the rats getting the ration containing 30 per cent of the dried beef is somewhat higher than that of the other group, but the growth of all the rats is far below normal. Ophthalmia developed in six of the rats.

Thus far it appears that none of the samples of dried beef tested, even when used to the extent of 30 per cent of the ration, furnished sufficient vitamin A for normal growth in young rats. In Figure 8, however, are shown the growth curves of a group of rats that received 50 per cent of dried beef Nos. 1018, 1021, and 1024 as the sole source of vitamin A and which made practically normal growth. Rat No.

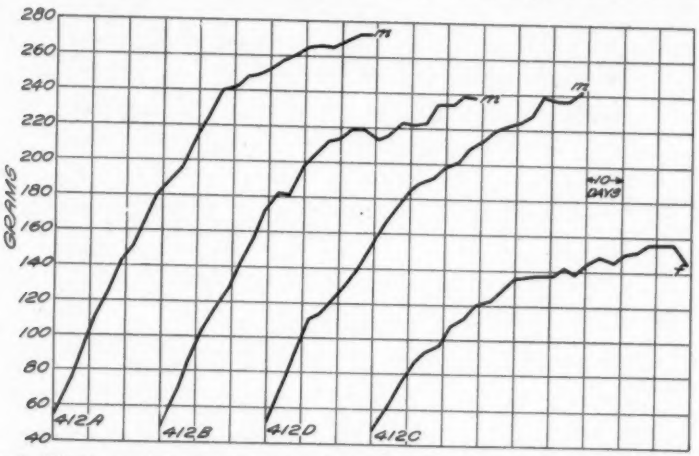


FIG. 8.—Vitamin A in beef. Graphs showing rate of growth of rats that were fed a ration containing 50 per cent of dried round steak as the source of vitamin A in an otherwise adequate diet. The ration was made up in parts by weight as follows: Dried beef Nos. 1018, 1021, and 1064, 50; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 2; cassava starch, 34; total, 100

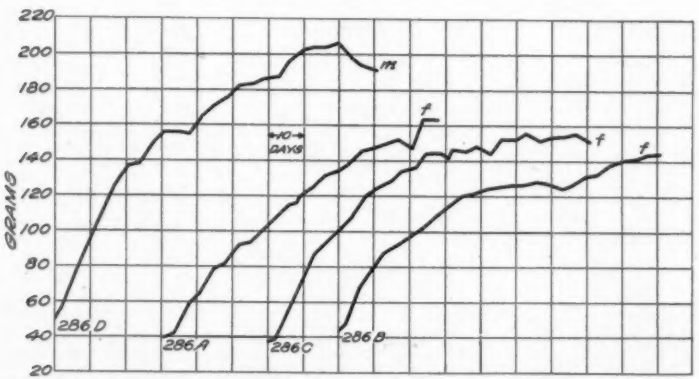


FIG. 9.—Vitamin A in beef. Graphs showing rate of growth of rats that were fed a ration containing 95 per cent of dried round steak as the source of vitamins A and B and of protein. The ration was made up in parts by weight as follows: Dried beef, 95; calcium carbonate, 4.5; sodium chloride, 0.5; total, 100

412 A, a male, made fully normal growth and was in fair condition at the end of the test. The other male rats, Nos. 412 B and 412 D, did not make quite so rapid growth and were in fair condition at the close of the experiment. The female rat, No. 412 C, did not make quite normal growth and was in fair condition at the end.

In Figure 9 are shown the growth curves of rats that were fed a ration containing 95 per cent of dried beef as the sole source of vitamins A and B and of protein. During the course of the test samples of beef Nos. 869, 874, 902, 906, 912, and 934, which also had been used in the tests already reported, and in addition sample No. 957, were used. The rats made nearly, though not quite, normal growth, but it can not be stated positively that lack of vitamin A was the limiting factor, since no vitamin B was added to the ration. All the rats were in good condition at the end of the test.

VITAMIN A IN PORK

Description of Samples

The pork consisted of six lots of pork loins and one lot of frozen tenderloins. Each lot of pork loins consisted of three or four loins weighing 6 to 8 pounds each. One lot had its origin in South St. Paul, one in Omaha, two in Chicago, and two in Washington, D. C. The lot of tenderloins consisted of 12 pieces weighing slightly less than 1 pound each. The origin of this product is not known. The above-described meat was purchased in Washington from local packers and from the branch houses of western packers.

The pork loins were boned out and the lean meat was separated as completely as practicable from fat and connective tissue, and was ground and dried in the manner previously described for beef. The tenderloins were trimmed free from visible fat and were dried in like manner. Each sample of dried pork was analyzed for nitrogen and fat before being used in a ration. Feeding tests were begun promptly after the meat had been dried.

Feeding Tests with Dried Pork Loins

In Figure 10 are shown the growth curves of two groups of rats that were fed rations containing 15 and 30 per cent, respectively, of dried pork loin No. 873 as the sole source of vitamin A. Of the group receiving the ration that contained 15 per cent of dried pork loin, three rats made but little more growth than might have been expected on account of the store of vitamin A in their tissues, while the fourth rat made fair growth for 30 days, when it died.

Rats Nos. 206 A to 206 D, inclusive, that were fed a ration containing 30 per cent of the same lot of dried pork, made somewhat better growth than the first three rats in the preceding group, but all finally declined in weight, three developed ophthalmia, and the fourth died.

In Figure 11 are shown the growth curves of two groups of rats that were fed rations containing 20 and 30 per cent, respectively, of dried pork loin No. 884 as the sole source of vitamin A. Of the rats that were fed the ration containing 20 per cent of dried pork, three made fair growth but the other did not do so well. All finally declined in weight, two rats developing ophthalmia.

Rats Nos. 228 A to 228 D, inclusive, that were fed the ration containing 30 per cent of the same lot of dried pork, made somewhat better growth, but nutritive failure finally resulted. Three rats in this group developed ophthalmia and the fourth died.

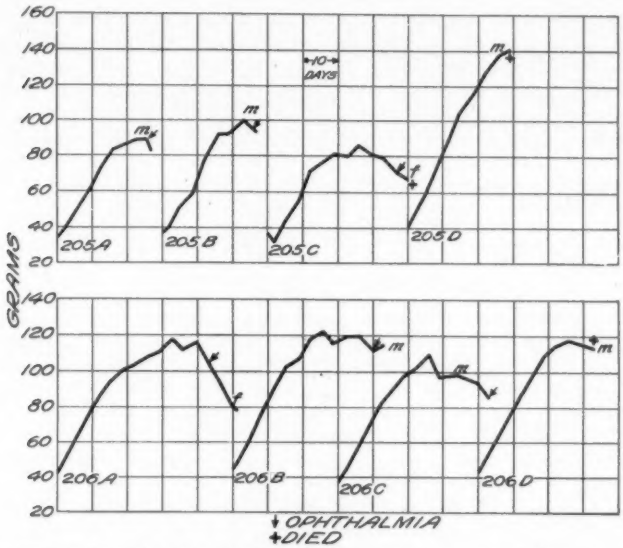


FIG. 10.—Vitamin A in pork. Graphs showing rate of growth of two groups of rats that were fed rations containing 15 and 30 per cent, respectively, of the dried lean meat from pork loins as the source of vitamin A in otherwise adequate diets

The ration fed to rats Nos. 205 A to 205 D, inclusive, was made up in parts by weight as follows: Dried pork loins No. 873, 15; purified ox muscle, 10.9; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 7.9; cassava starch, 52.2; total, 100

The ration fed to rats Nos. 206 A to 206 D, inclusive, was made up as follows: Dried pork loin No. 873, 30; purified ox muscle, 1.9; ash mixture, 4; hardened cottonseed oil, 4.3; cassava starch, 59.8; total, 100

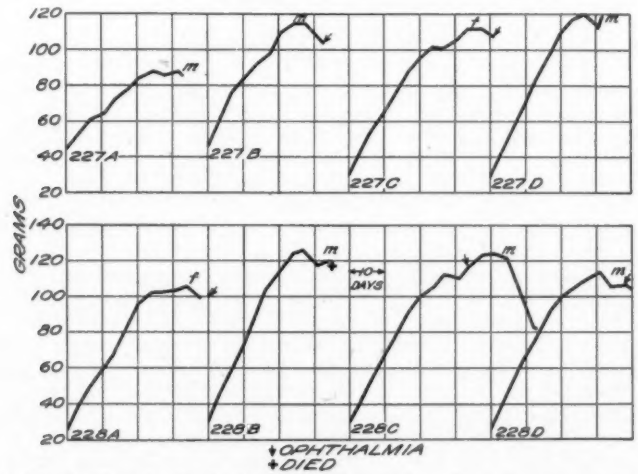


FIG. 11.—Vitamin A in pork. Graphs showing rate of growth of two groups of rats that were fed rations containing 20 and 30 per cent, respectively, of the dried lean meat from pork loins as the source of vitamin A in otherwise adequate diets

The ration fed to rats Nos. 227 A to 227 D, inclusive, was made up in parts by weight as follows: Dried pork loins No. 884, 20; purified ox muscle ,10.2; dried baker's yeast, 5; ash mixture, 4; hardened cotton seed oil, 1.1; cassava starch, 59.7; total, 100

The ration fed to rats Nos. 228 A to 228 D, inclusive, was made up as follows: Dried pork No. 884, 30; purified ox muscle, 5.3; dried baker's yeast, 5; ash mixture, 4; cassava starch, 55.7; total, 100

In Figure 12 are shown the growth curves of two groups of rats that were fed rations containing 15 and 30 per cent, respectively, of dried pork loin No. 903 as the sole source of vitamin A. Rats Nos. 242 A to 242 D, inclusive, that were fed the ration containing 15 per

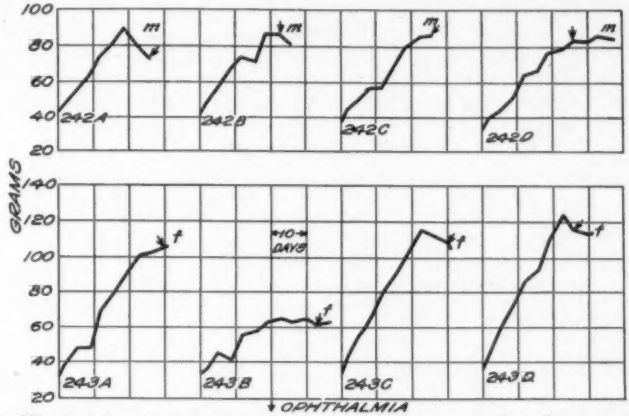


FIG. 12.—Vitamin A in pork. Graphs showing rate of growth of two groups of rats that were fed rations containing 15 and 30 per cent, respectively, of dried lean meat from pork loins as the source of vitamin A in otherwise adequate diets

The ration fed to rats Nos. 242 A to 242 D, inclusive, was made up in parts by weight as follows: Dried pork loin No. 903, 15; purified ox muscle, 13.2; dried brewer's yeast, 5; ash mixture, 4; cassava starch, 62.8; total, 100

The ration fed to rats Nos. 243 A to 243 D, inclusive, was made up as follows: Dried pork loin No. 903, 30; purified ox muscle, 6.4; dried brewer's yeast, 5; ash mixture, 4; cassava starch, 54.6; total, 100

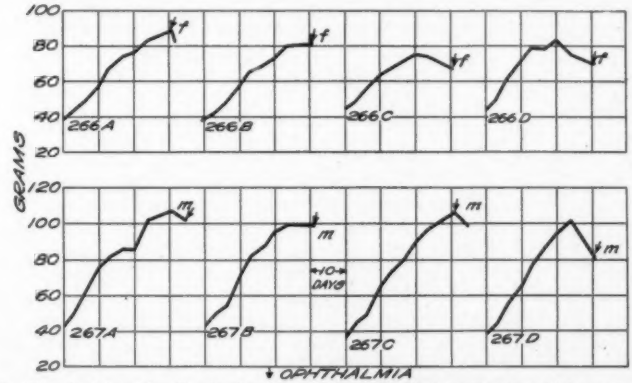


FIG. 13.—Vitamin A in pork. Graphs showing rate of growth of two groups of rats that were fed rations containing 20 and 40 per cent, respectively, of the dried lean meat from pork loins as the source of vitamin A in otherwise adequate diets

The rations fed to rats Nos. 266 A to 266 D, inclusive, was made up in parts by weight as follows: Dried pork loin No. 920, 20; purified ox muscle, 5.4; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 6.8; cassava starch, 53.8 total, 100

The ration fed to rats Nos. 267 A to 267 D, inclusive, was made up as follows: Dried pork loin No. 920, 40; ash mixture, 4; hardened cottonseed oil, 3.6; cassava starch, 52.4; total, 100

cent of dried pork, did not make much growth and all developed ophthalmia. Three of the rats that were fed the ration containing 30 per cent of dried pork made fair growth, reaching weights between 106 and 124 grams, while the fourth made only slight growth, but all the rats in this group developed ophthalmia.

The growth curves of two groups of rats that were fed rations containing 20 and 40 per cent, respectively, of dried pork loin No. 920 as the source of vitamin A are shown in Figure 13. Rats Nos. 266 A to 266 D, inclusive, that were fed the ration containing 20 per cent of dried pork, made poor growth and all developed ophthalmia, while rats Nos. 267 A to 267 D, inclusive, that received the ration containing 40 per cent of dried pork, made slightly better growth, reaching weights between 100 and 106 grams. All the rats in the two groups developed ophthalmia by the thirty-fifth day of the test.

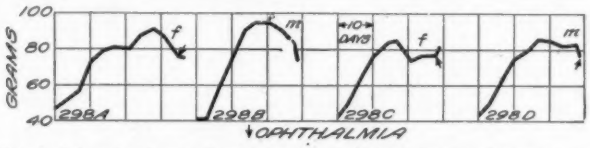


FIG. 14.—Vitamin A in pork. Graphs showing rate of growth of rats that were fed a ration containing 40 per cent of dried lean meat from pork loins as the source of vitamin A in an otherwise adequate diet. The ration was made up in parts by weight as follows: Dried pork loin No. 938, 40; ash mixture, 4; cassava starch, 56; total 100

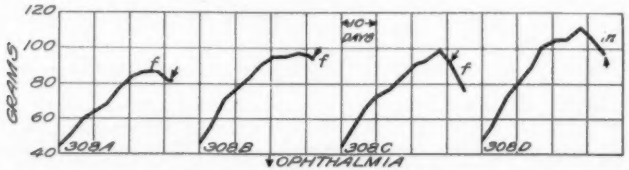


FIG. 15.—Vitamin A in pork. Graphs showing rate of growth of rats that were fed a ration containing 40 per cent of dried lean meat from pork loins as the source of vitamin A in an otherwise adequate diet. The ration was made up in parts by weight as follows: Dried pork loin No. 907, 40; ash mixture, 4; cassava starch, 56; total, 100

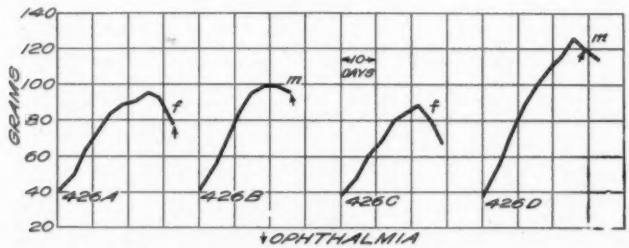


FIG. 16.—Vitamin A in pork. Graphs showing rate of growth of rats that were fed a ration containing 50 per cent of dried pork tenderloin as the source of vitamin A in an otherwise adequate diet. The ration was made up in parts by weight as follows: Dried pork tenderloin No. 1024, 50; ash mixture, 4; cassava starch, 46; total, 100

In Figure 14 are shown the growth curves of rats that were fed a ration containing 40 per cent of dried pork loin No. 938 as the source of vitamin A. The rats made poor growth and all soon declined in weight and developed ophthalmia.

In Figure 15 are shown the growth curves of rats that were fed a ration containing 40 per cent of dried pork loin No. 907 as the source of vitamin A. These rats made only slightly better growth than those shown in Figure 14 and all soon declined in weight and developed ophthalmia.

In Figure 16 are shown the growth curves of rats that were fed a ration containing 50 per cent of dried pork tenderloin No. 1024 as the sole source of vitamin A. Three of the rats made poor growth, while the fourth did somewhat better, but all finally declined in weight, and three of the rats developed ophthalmia.

In Figure 17 are shown the growth curves of rats that were fed a ration consisting of 95 per cent dried pork loins, 4.5 per cent calcium carbonate, and 0.5 per cent sodium chloride. Three lots of pork loins Nos. 873, 884, and 903, that had been used in experiments already reported, were fed during the course of the experiment. While this group of rats made much better growth than any of the other groups that were fed rations containing dried pork as a source of vitamin A, they did not make nearly normal growth and all developed ophthalmia.

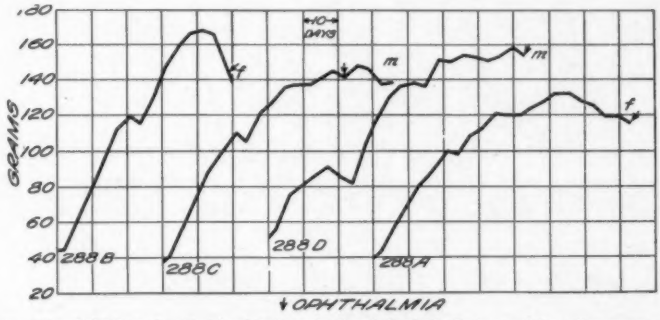


FIG. 17.—Vitamin B in pork. Graphs showing rate of growth of rats that were fed a ration containing 95 per cent of dried lean meat from pork loins as the source of vitamins A and B and of protein. The ration was made up in parts by weight as follows: Dried pork loins, samples Nos. 873, 884, and 903, 95; calcium carbonate, 4.5; sodium chloride, 0.5; total, 100

VITAMIN A IN LAMB

DESCRIPTION OF SAMPLES

Six samples of lamb were tested for vitamin A. Each of five samples was prepared from the hind saddle of the carcass of high-grade, heavy, fat lamb, while the other sample was prepared from two hind saddles of lighter weight fat carcasses. The hind saddles of the heavy lambs weighed from 18 to 20 pounds each, while the lighter saddles weighed 12 pounds each. One lamb carcass had its origin in East St. Louis, Ill., one in Omaha, Nebr., one in South St. Joseph, Mo., and the other three lambs were slaughtered at Benning, D. C. The muscle tissue was separated from fat and connective tissue and dried in the manner previously described for beef. Each sample of dried lamb was analyzed for nitrogen and fat before being incorporated in a ration.

FEEDING TESTS WITH LAMB

In Figure 18 are shown the growth curves of rats that were fed a ration containing 30 per cent of dried lamb No. 870 as the sole source of vitamin A in an otherwise adequate diet. Each of the rats made very good growth for a time, but all finally ceased to gain, then lost weight, and developed ophthalmia. One female rat, No. 201 C, nearly reached normal weight and then declined and developed oph-

thalmia. This sample of dried lamb is richer in vitamin A than any of the samples of dried beef or pork which have been reported in this paper.

In Figure 19 are shown the growth curves of two groups of rats that were fed rations containing 15 and 30 per cent, respectively, of dried lamb No. 876 as the source of vitamin A. Neither group of rats made much growth, all finally declined in weight, and six of the eight rats developed ophthalmia.

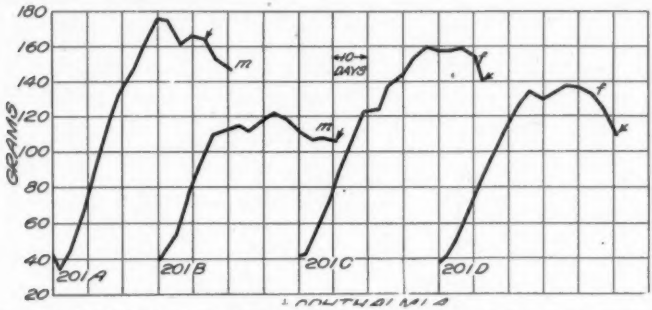


FIG. 18.—Vitamin A in lamb. Graphs showing rate of growth of rats that were fed a ration containing 30 per cent of dried lamb as the source of vitamin A in an otherwise adequate diet. The ration was made up in parts by weight as follows: Dried lamb No. 870, 30; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 3.5; cassava starch, 52.5; total, 100

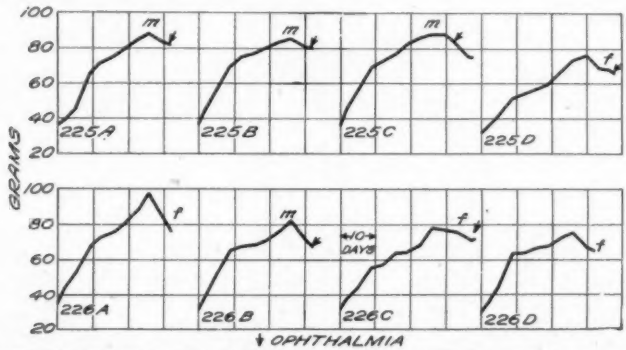


FIG. 19.—Vitamin A in lamb. Graphs showing rate of growth of two groups of rats that were fed rations containing 15 and 30 per cent, respectively, of dried lamb as the source or vitamin A in an otherwise adequate diet

The ration fed to rats Nos. 225 A to 225 D, inclusive, was made up in parts by weight as follows: Dried lamb No. 876, 15; purified ox muscle, 10.5; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 6.1; cassava starch, 54.4; total, 100

The ration fed to rats Nos. 226 A to 226 D, inclusive, was made up as follows: Dried lamb No. 876, 30; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 2.1; cassava starch, 53.9; total, 100

The growth curves of two groups of rats that were fed rations containing 20 and 30 per cent, respectively, of dried lamb No. 897 are shown in Figure 20. Both groups of rats made poor growth, only little if any better than that made by rats fed a basal ration free from vitamin A. Every rat developed opthalmia in 22 days or less.

In Figure 21 are shown the growth curves of two groups of rats that were fed rations containing 15 and 30 per cent, respectively, of dried lamb No. 933 as the source of vitamin A. With the exception

of one rat, No. 293 B, which made only fair growth and then declined in weight, the rats that were fed these rations made very poor growth. Six of the eight rats developed ophthalmia.

In Figure 22 are shown the growth curves of rats that were fed a ration containing 20 per cent of dried lamb No. 992 as the source of vitamin A. All the rats made excellent growth, practically

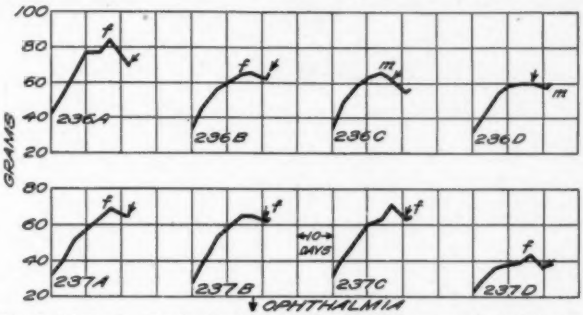


FIG. 20.—Vitamin A in lamb. Graphs showing rate of growth of two groups of rats that were fed rations containing 20 and 30 per cent, respectively, of dried lamb as the source of vitamin A in otherwise adequate diets

The ration fed to rats Nos. 236 A to 236 D, inclusive, was made up in parts by weight as follows: Dried lamb No. 897, 20; purified ox muscle, 7.9; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 4.6; cassava starch, 53.5; total, 100

The ration fed to rats Nos. 237 A to 237 D, inclusive, was made up as follows: Dried lamb No. 897, 30; purified ox muscle, 1.8; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 2; cassava starch, 52.2; total, 100

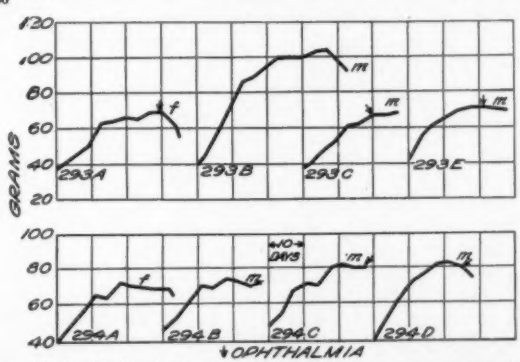


FIG. 21.—Vitamin A in lamb. Graphs showing rate of growth of two groups of rats that were fed rations containing 15 and 30 per cent, respectively, of dried lamb as the source of vitamin A in otherwise adequate diets.

The ration fed to rats Nos. 293 A to 293 E, inclusive, was made up in parts by weight as follows: Dried lamb No. 933, 15; purified ox muscle, 9; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 7.7; cassava starch, 54.3; total, 100

The ration fed to rats Nos. 294 A to 294 D, inclusive, was made up in parts by weight as follows: Dried lamb No. 933, 30; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 6.6; cassava starch, 49.4; total, 100

normal for the sexes. One male, No. 372 C, reached the maximum weight for the group and then rapidly declined in weight to 181 grams when it was removed from the test. This rat was in very poor condition at the time but showed no signs of ophthalmia. The three other rats gained in weight until the close of the test and were in excellent condition at the end. This sample of dried lamb is richer in vitamin A than any of the other samples of meat thus far reported in this paper.

In Figure 23 are shown the growth curves of rats that were fed a ration containing 20 per cent of dried lamb No. 994 as the source of vitamin A. The two male rats in the group made remarkably good

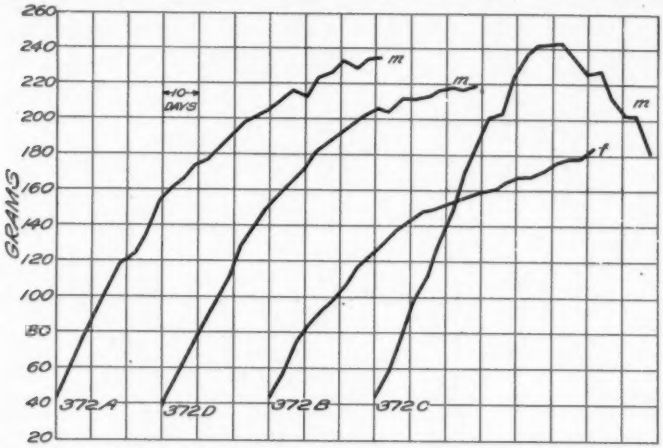


FIG. 22.—Vitamin A in lamb. Graphs showing rate of growth of rats that were fed a ration containing 20 per cent of dried lamb as the source of vitamin A in an otherwise adequate diet. The ration was made up in parts by weight as follows: Dried lamb No. 992, 20; purified casein, 10.6; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 3; cassava starch, 52.4; total, 100

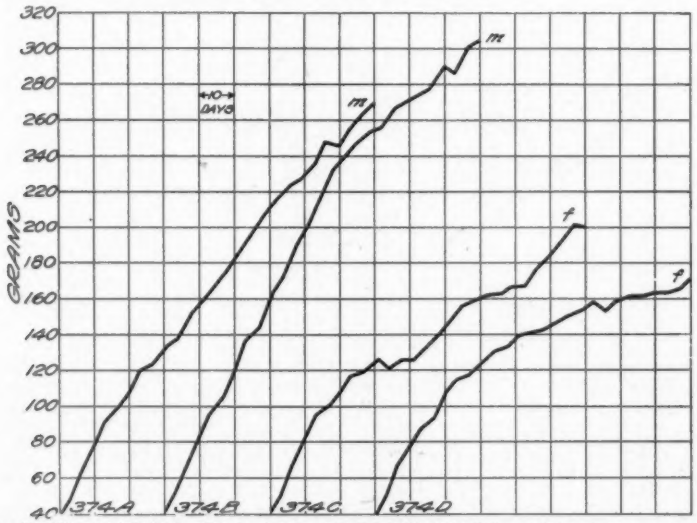


FIG. 23.—Vitamin A in lamb. Graphs showing rate of growth of rats that were fed a ration containing 20 per cent of dried lamb as the source of vitamin A in an otherwise adequate diet. The ration was made up in parts by weight as follows: Dried lamb No. 994, 20; purified casein, 9.8; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 3.3; cassava starch, 52.9; total, 100

growth, while the two females did nearly as well, sex considered. With one exception, the rats were still gaining in weight when the test was discontinued at the end of 90 days, and all were in excellent condition at that time.

This sample of dried lamb is even richer in vitamin A than the preceding sample (No. 992, fig. 22). It is interesting to record that the two hind saddles of lamb from which samples Nos. 992 and 994 were prepared were purchased at the same time from a local meat-packing establishment. On inquiry it was found that the two lamb carcasses probably came out of a carload of lambs purchased in Chicago and slaughtered at Benning, D. C. No information was available as to how the lambs had been fed.

SUMMARY OF RESULTS AND CONCLUSIONS

Since the rats fed the basal rations alone made a certain amount of growth for a time, due, no doubt, principally to the store of vitamin A in their tissues, in interpreting the results of any experiment allowance must be made for the growth made by rats fed the basal ration. Unfortunately, as yet, a satisfactory unit for measuring the vitamin A content of a food product is lacking, so that the values obtained are reported only in very general terms. Since the energy values of the different rations are practically the same, the growth value obtained for one ration may be compared directly with that obtained from another.

VITAMIN A IN BEEF

A total of 10 samples of beef, representing a like number of cattle, were tested for their vitamin A content by means of feeding tests with 11 groups of rats of 4 members each, or a total of 44 rats. Six samples of dried beef Nos. 869, 874, 902, 906, 912, and 934 were used in the proportions either of 15 or 30 per cent in rations fed to 9 groups of rats. The results of these tests show that none of those samples of dried beef, in the quantities fed, furnished sufficient vitamin A to induce normal growth in young rats. The best growth was made by rats fed rations containing 30 per cent, respectively, of samples of beef Nos. 869, 874, and 934, as shown in Figures 2, 3, and 7.

When the proportion of dried meat in the ration was increased to 50 per cent, much more satisfactory results were obtained. A group of four rats was fed a ration containing 50 per cent of dried beef, samples Nos. 1018, 1021, and 1024 being used in succession. One male rat made excellent growth, two other male rats did nearly as well, and a female rat made nearly normal growth (fig. 8). All four rats were in fair condition at the close of the test. Apparently 50 per cent of these samples of dried beef in a ration furnished nearly sufficient vitamin A for normal growth.

Another group of rats was fed a ration containing 95 per cent of dried beef, the same samples that were used in the tests reported in Figures 2 to 7, inclusive, being used in succession; and, in addition, another lot of beef, No. 957 (fig. 9). The rats in this group, one male and three females, made nearly, but not quite, normal growth, and all were in good condition at the end of test.

The results of these tests show that the samples of beef tested were relatively poor in vitamin A, but that when used in rations in proportions of 50 or 95 per cent, nearly sufficient vitamin A for normal growth was furnished.

VITAMIN A IN PORK

Six lots of pork loins, representing 18 hogs, and 1 lot of pork tenderloins, representing 12 hogs, were tested for their content of vitamin A by means of feeding tests with 12 groups of 4 rats each, or a total of 48 rats. In general, the results obtained with dried fresh pork as a source of vitamin A are less satisfactory than those obtained with beef. The rations tested contained dried pork in percentages ranging from 15 to 95 per cent, but in no instance did a ration furnish sufficient vitamin A to meet the requirements of growing rats. Fair growth was made by a number of rats that were fed rations containing 30 to 40 per cent of dried pork (figs. 10, 11, and 12) and by a few rats receiving a ration containing 20 per cent of dried pork (fig. 11), but even 50 per cent of dried pork tenderloin in a ration proved to be inadequate as a source of vitamin A (fig. 16). A ration containing 95 per cent of dried pork loins did not furnish sufficient vitamin A to induce normal growth in young rats (fig. 17). Four rats were fed this ration, and, although they grew fairly well for a time, all finally declined in weight and developed ophthalmia.

The results of the experiments with fresh pork show that the samples tested were relatively poor in vitamin A.

VITAMIN A IN LAMB

A total of 6 samples of lamb, representing 7 animals, were tested for their vitamin A content by means of feeding tests with 9 groups of rats of 4 members each, or a total of 36 rats. There were rather wide differences in the vitamin A content of the several samples of lamb. Samples Nos. 876, 897, and 933 were poorer in vitamin A than the others. Each of these samples was used in the proportions of 15 and 30 per cent in rations, but none of the rats made much growth (figs. 19, 20, 21).

Sample of lamb No. 870 was somewhat richer in vitamin A, and rats that were fed a ration containing 30 per cent of this sample made considerable growth, although they finally declined in weight and developed ophthalmia (fig. 18). One male rat reached a maximum weight of 176 grams in 30 days, and a female a maximum weight of 160 grams in 37 days.

Samples of lamb No. 992 and 994 were the richest in vitamin A of any of the samples of beef, pork, or lamb that are reported in this paper. The four rats fed the ration containing 20 per cent of sample No. 992 made normal growth, and three of the rats were still gaining in weight at the end of the 90-day test, but the fourth rat, a male, declined rapidly in weight after having reached a maximum weight of 243 grams in 53 days (fig. 22). The rats that were fed a ration containing 20 per cent of the other sample of lamb, No. 994, made even better growth. The two male rats in this group attained weights of 268 and 304 grams, respectively, in 90 days, while the females weighed 171 and 200 grams, respectively, at the end of the same period (fig. 23).

The results of the vitamin A tests with lamb show that three of the six samples examined were rather poor in vitamin A, one contained a fair proportion, while the remaining two samples were richer in vitamin A than any of the other samples of meat that are reported in this paper.

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THE RELATIONSHIP BETWEEN THE WEIGHT OF EGGS AND THE WEIGHT OF CHICKS ACCORDING TO SEX [1]

By M. A. JULL, *Poultry Husbandman*, and J. P. QUINN, *Chief Scientific Aid*, *Bureau of Animal Industry, United States Department of Agriculture*

INTRODUCTION

In the domestic fowl the relationship between the weight of eggs and the weight of chicks according to sex at hatching time is an interesting problem, inasmuch as the mature male is normally heavier than the mature female. If the chicks of both sexes weigh practically the same at hatching time, then there remains an interesting study of different rates of growth of the sexes. On the other hand, if the weights of the sexes are significantly different at hatching time, it is of importance, then, to try to discover why the weights are different and whether the sexes of standard-bred chicks can be separated according to differences in weight.

This study was undertaken to determine, first, the relationship between the weights of chicks of the two sexes at hatching time; and, second, the relationship between the weights of the eggs and the weights of the chicks hatched from them.

PROCEDURE

The eggs used in this study were obtained from four different sources: 53 Rhode Island Red yearling hens, 30 Rhode Island Red pullets, 50 Barred Plymouth Rock yearling hens, and 113 Barred Plymouth Rock pullets. All the females were mated to Rhode Island Red cockerels. The eggs were saved from March 15 to March 23, when the incubators were set. Only a portion of the Rhode Island Red eggs laid during the period mentioned were used in this study. All the eggs laid by the Barred Plymouth Rocks were used. In this paper no account is taken of the infertile eggs, embryos which died during the period of incubation, and chicks which died in shell at hatching time. The eggs were weighed daily as laid, the weights being recorded to hundredths of a gram. The chicks were weighed at hatching time, the weights also being recorded to hundredths of a gram.

The sex of the chicks from the Barred Plymouth Rock females was recorded at hatching time, and that of the Rhode Island Red chicks when the chicks were 9 weeks old. Distinguishing the sex of chicks from the Barred Plymouth Rock females mated to Rhode Island Red males was an easy matter, since the sex-linked barring pattern of the barred females is transmitted to the sons only. The male chicks always have the white spot on the top of the head and yellow shanks, characteristic of purebred Barred Plymouth Rock male chicks, while the female chicks of this cross lack the white spot and have black or very dark shanks.

[1] Received for publication Oct. 25, 1924; issued September, 1925.

EXPERIMENTAL DATA

In Table I is shown, for each of the four groups of birds, the relationship between the weight of eggs from which males were hatched and the weight of the eggs from which females were hatched. In the case of the eggs from the Barred Plymouth Rock yearling hens, those giving rise to males have a slightly lower mean weight than the eggs giving rise to females, but in the other three cases the reverse is true. In no case, however, is the difference in weights significant, the differences with their probable errors being 0.34 ± 0.55, 0.60 ± 0.42, 0.44 ± 0.48, and 0.48 ± 0.34 gm.

This situation is in harmony with the results obtained by the senior writer [2] in the case of eggs from 30 Barred Plymouth Rock pullets mated to Brown Leghorn males, and by both authors [3] in the case of eggs from 153 Barred Plymouth Rock females mated to Rhode Island Red males. It seems, then, that selection of hatching eggs according to weight can not be expected to affect the sex ratio of chicks hatched therefrom.

TABLE I.—*Relationship between the mean weight of eggs producing males and the mean weight of eggs producing females in each of four groups; and the relationship of the mean weight of eggs producing males and females, respectively, between the yearling hen and pullet groups of each breed*

Group	Number	Mean weight of eggs from which males were hatched	Number	Mean weight of eggs from which females were hatched	Difference
		Gm.		*Gm.*	
Rhode Island Red yearlings	66	59.88±0.36	49	59.54±0.41	0.34±0.55
Rhode Island Red pullets	59	56.61± .30	54	56.01± .30	.60± .42
Difference		3.27± .47		3.53± .51	
Barred Plymouth Rock yearlings	63	59.91± .30	49	60.35± .37	.44± .48
Barred Plymouth Rock pullets	123	57.93± .24	106	57.45± .24	.48± .34
Difference		1.98± .38		2.90± .44	

Table I shows, for each of the two breeds, in the case of eggs giving rise to males and females, respectively, the difference in weights of eggs from yearling hens and from pullets. In both breeds the eggs from yearling hens were considerably heavier than the eggs from pullets. In the Rhode Island Reds, the difference in the weights of eggs giving rise to males is 3.27 ± 0.47 gm. in favor of the eggs from the yearling hens, and the difference in the weights of eggs giving rise to females is 3.53 ± 0.51 gm. in favor of the yearling hens. In the Barred Plymouth Rocks, the difference in the weights of eggs giving rise to males is 1.98 ± 0.38 gm. in favor of the yearling hens, and the difference in the weights of eggs giving rise to females is 2.90 ± 0.44 gm. In each case, the difference is considerably more than three times its probable error, and it is seen, then, that in both breeds the yearling hens laid significantly heavier eggs than the pullets.

The weights of the chicks of each sex from each of the four groups of birds are shown in Table II. In each of the four groups the mean weight of the females is lighter than that of the males, but in no

[2] JULL, M. A. THE RELATION OF ANTECEDENT EGG PRODUCTION TO THE SEX RATIO OF THE DOMESTIC FOWL. Jour. Agr. Research 28: 199–224. 1924.

[3] JULL, M. A., and QUINN, J. P. THE SHAPE AND WEIGHT OF EGGS IN RELATION TO THE SEX OF CHICKS IN THE DOMESTIC FOWL. Jour. Agr. Research 29: 195–202. 1924.

case is the difference significant, the differences with their probable errors being 0.63 ± 0.43, 0.81 ± 0.32, 0.11 ± 0.43, and 0.68 ± 0.25 gm. These results would indicate that chicks can not be assorted by sex at hatching time according to their weights.

TABLE II.—*Relationship between the mean weight of male chicks and the mean weight of female chicks, in each of four groups; and the relationship of the mean weight of male and female chicks, respectively, between the yearling hen and the pullet groups of each breed*

Group	Number	Mean weight of male chicks	Number	Mean weight of female chicks	Difference
		Gm.		*Gm.*	
Rhode Island Red yearlings	66	39.72±0.31	49	39.09±0.30	0.63±0.43
Rhode Island Red pullets	59	37.68± .23	54	36.87± .23	.81± .32
Difference		2.04± .39		2.22± .38	
Barred Plymouth Rock yearlings	63	39.07± .28	49	38.96± .33	.11± .43
Barred Plymouth Rock pullets	123	37.99± .18	106	37.31± .18	.68± .25
Difference		1.08± .33		1.65± .38	

Table II also shows the relationship between the weights of males and females, respectively, obtained from eggs laid by the yearling hens and from eggs laid by the pullets for each of the two breeds. In the case of the Rhode Island Reds, the difference in the mean weight of males from eggs laid by the yearling hens and from eggs laid by pullets is 2.04 ± 0.39 gm. in favor of the yearling hens, and the difference in the mean weight of females from eggs laid by yearling hens and from eggs laid by pullets is 2.22 ± 0.38 gm. in favor of the yearling hens. In the case of the Barred Plymouth Rocks, the difference in the mean weight of males from eggs laid by the yearling hens and from eggs laid by the pullets is 1.08 ± 0.33 gm., and the difference in the mean weight of females from eggs laid by yearling hens and from eggs laid by pullets is 1.65 ± 0.38 gm. The differences are significant in every case, and are what would naturally be expected in view of the significant differences in the mean weights of eggs laid by the yearling hens and by the pullets, as shown in Table I.

It might be possible, however, for chicks from eggs laid by pullets to be as heavy or even heavier than chicks from eggs laid by yearling hens, if the chicks from pullet eggs constitute a higher mean percentage of the mean egg weight than the chicks from yearling-hen eggs. Such a situation might be true whether the mean weight of the pullet eggs was as great or even less, as in the case of this study, than the mean weight of the yearling-hen eggs. In other words, do all chicks, whether they come from eggs laid by yearling hens or from eggs laid by pullets tend to constitute the same percentage of the weight of the eggs from which they were hatched?

The data in Table III show, for each of the four groups of birds and for the sexes, respectively, the chick weight times one hundred over the egg weight or the mean percentage chick weight of egg weight.

The female chicks constitute a lower mean percentage of the eggs from which they were hatched in all four cases. In no case, however, is the difference in mean percentage significant, the differences with their probable errors being: 0.68 ± 0.28, 0.73 ± 0.33, 0.60 ± 0.35, and 0.61 ± 0.30. It would seem, then, that in the case of neither

yearling hens nor pullets is there any significant difference in the mean percentage male and female chick weight of egg weight.

TABLE III.—*Relationship between the mean percentage chick weight of egg weight in eggs producing males and the mean percentage chick weight of egg weight in eggs producing females, in each of four groups; and the relationship between the mean percentage chick weight of egg weight in eggs producing males and females, respectively, in each breed*

Group	Number	Mean percentage chick weight of egg weight in eggs from which males were hatched	Number	Mean percentage chick weight of egg weight in eggs from which females were hatched	Difference
Rhode Island Red yearlings	66	66.33±0.19	49	65.65±0.21	0.68±0.28
Rhode Island Red pullets	59	66.56± .25	54	65.83± .22	.73± .33
Difference		.23± .31		.18± .30	
Barred Plymouth Rock yearlings	63	65.16± .23	49	64.56± .27	.60± .35
Barred Plymouth Rock pullets	123	65.60± .20	106	64.99± .23	.61± .30
Difference		.44± .30		.43± .35	

When the mean percentage chick weight of egg weight per sex between the yearling hens and pullets is considered, it is seen that in no case was there a significant difference. In every case, the mean percentage was greater in the case of the pullets than in the case of the yearlings. This is accounted for in the fact that the mean weight of the pullet eggs was somewhat lower than the mean weight of the yearling-hen eggs. The results in Table III tend to show that pullet eggs having the same weight as yearling-hen eggs tend to produce chicks having as great a mean weight as the chicks from eggs laid by yearling hens. In other words, the weight of the chick seems to be determined, more or less, by the weight of the egg from which it was hatched. The mean weight of the chicks hatched from small eggs, whether they be from yearlings or pullets, seems to be reduced according to the extent that the small eggs are used in incubation.

CONCLUSIONS

There is no significant difference between the weight of yearling-hen eggs from which males are hatched and the weight of yearling-hen eggs from which females are hatched.

There is no significant difference between the weight of pullet eggs from which males are hatched and the weight of pullet eggs from which females are hatched.

There is no significant difference in the weight of male chicks and the weight of female chicks from eggs laid by yearling hens.

There is no significant difference in the weight of male chicks and the weight of female chicks from eggs laid by pullets.

There is no significant difference in the percentage chick weight (in either sex) of egg weight in yearling hens as compared with pullets.

If pullet eggs have a significantly lower mean weight than yearling-hen eggs, the chicks hatched from pullet eggs will also tend to have a significantly lower mean weight than the chicks hatched from yearling-hen eggs.

The separation of the sexes of chicks at hatching time on the basis of weight is unreliable.

TESTS OF DEEP-WELL TURBINE PUMPS[1]

By F. L. Bixby

Senior Irrigation Engineer, Division of Agricultural Engineering, Bureau of Public Roads, United States Department of Agriculture

INTRODUCTION

The deep-well turbine pump is a modification of the vertical centrifugal pump so designed as to obviate the necessity of constructing a pit where the depth to water is beyond the practical limit for installing centrifugal pumps. It is built up in vertical units or bowls, one above another. Any number of bowls may be used, the number varying according to the head to be pumped against and the discharge desired, 12 to 30 feet being allowed for each stage or bowl. Each bowl contains a runner with guide or diffusion vanes. The vertical shaft extends through all the bowls, and the runners are attached to the vertical shaft. The multiplicity of runners and bowls gives a booster effect to the pump and decreases the speed at which it would be necessary to operate it if but one runner were used. The pump is entirely submerged. It is primed automatically, and oiling of the bearing is accomplished either through an oil pipe which incloses the shafting or by means of small pipes leading from an oil supply at the surface and directly attached to the bearings. Where the shaft and bearings are inclosed in an oil pipe they are protected from wear caused by sand pumped out in the discharge water.

This type of pump is adapted to pumping conditions which involve a depth to the water level of more than 75 feet, or a variable water table below the 50-foot level. As new lands in the arid West in which the depth to water does not exceed 50 feet are rare, the practice of irrigation by pumping will inevitably call for the use of large numbers of deep-well turbine pumps, which meet all the requirements of irrigation up to the economic limit of pumping for the various crops that are grown.

PURPOSE OF TESTS

Used within the range of heads for which the deep-well turbine pump is suitable, the degree of success attending its use depends upon a proper regard for the economics of selection, installation, and operation. One of the most important factors in the economical operation of all types of pumps is their efficiency. Most operators entirely lose sight of this in the endeavor to produce the largest flow possible, regardless of the expenditure of power.

For a given head, there is a direct relation between the speeds of the pump and prime mover, respectively, which must be maintained by means of pulleys of proper sizes. Where pumps are direct-connected to motors or engines, the same factor relative to speed of pump and prime mover exists. Hence, knowing the height to which water is to be raised and the quantity to be pumped, it is possible

[1] Received for publication August 30, 1924; issued September, 1925.

to determine the size of pump needed and the speed at which it should be operated to give the most economical performance.

It was for the purpose of providing data of this kind, of which very little has hitherto been published, that the Division of Agricultural Engineering, Bureau of Public Roads, United States Department of Agriculture, conducted the tests which are reported here. The principal object was to determine the proper speed of pumps for various heads and capacities. In making the tests, a study of various types of impellers was not attempted, the aim being to obtain results which would conform with field installations.

THE PUMPS TESTED

The tests were made at the laboratory of the New Mexico Agricultural College, State College, N. Mex.[2] (fig. 1), under a cooperative agreement between the New Mexico Agricultural Experiment Station and the Division of Agricultural Engineering of the Bureau of Public Roads.

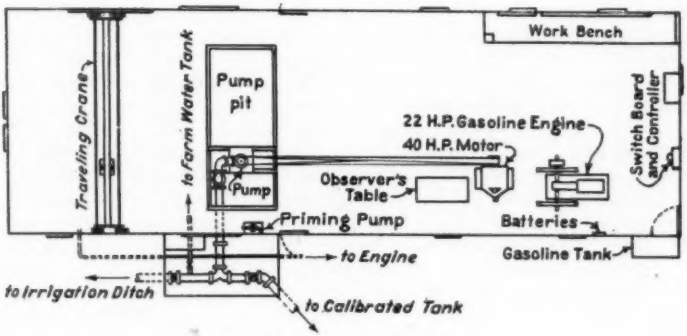


FIG. 1.—Plan of testing station

By the courtesy of three manufacturers, seven deep-well turbine pumps were obtained for testing. Pumps Nos. 1, 2, and 3 were obtained from the American Well Works, Aurora, Ill. They were 24-inch, 3-stage; 14-inch, 5-stage; and 12-inch, 7-stage, respectively. Pumps Nos. 4, 5, and 6, obtained from the Layne & Bowler Corporation of Los Angeles, were 24-inch, 2-stage; 17-inch, 4-stage; and 17-inch, 2-stage, respectively. Pump No. 7 was a 17-inch, 4-stage pump obtained from the Byron-Jackson Co. of San Francisco.

Each of these pumps was installed in a 12-inch bored and cased well with a capacity of 2,000 gallons per minute. Power was furnished by a 40-horsepower variable-speed motor with controller.

MODE OF PROCEDURE

Before the tests were started an investigation was made of the constancy of the voltage, and a prony brake test (fig. 2) was run on the motor to develop data for an efficiency curve which would con-

[2] Acknowledgment is due Dean W. Bloodgood, irrigation engineer, for his assistance in making pump tests, computation of data, and plotting original characteristic curves.

form with local operating conditions. Each pump was carefully installed, and all joints and connections were inspected before the tests started to insure efficient operation. The weir crest was checked for level, and the zero of the hook gauge was made to conform with the elevation of the crest of the weir.

Two men, observer and recorder, made the tests. Two sets of readings were taken separately for each observed head and these were averaged, unless there appeared to be too great a difference between readings, in which case a third set of readings was taken and the two between which there was the least difference were averaged.

The variations in head were controlled by throttling the discharge with a gate valve. Each set of tests started with the valve closed. For each succeeding run it was opened enough to give a decrement of 2 feet in head.

The cycle of readings taken by the observer was: Voltage, kilowatts, speed of motor, speed of pump, height of water on weir crest as indicated by hook gauge, and check reading on voltmeter and wattmeter. The readings taken by the recorder were: Vacuum head and pressure head, the latter being made at the time the wattmeter was read by the observer.

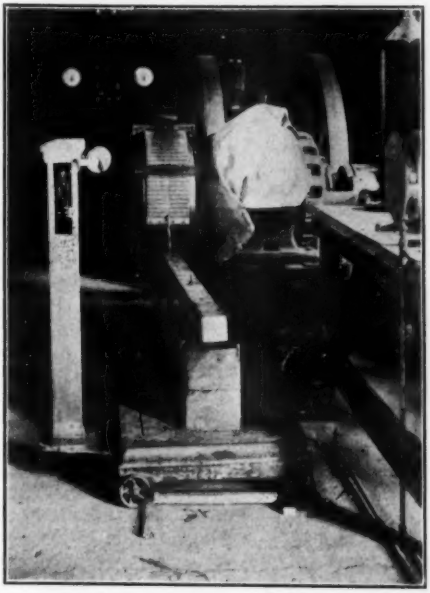


FIG. 2.—Prony brake test of motor

TEST DATA

The test data for each pump consist of tables giving complete results of the various average speeds and performance curves, including capacity-head curves, iso-efficiency curves, iso-brake-horsepower curves, and logarithmic curves showing the velocity-head difference between suction and discharge.

The tables give the speeds of the pump for varying capacities in gallons per minute and the corresponding pressure head, suction head, static head, velocity head, total head, brake horsepower, water horsepower, and efficiency. The pressure head and suction head were measured directly with mercury manometers. The static head was measured with a surveyor's level, being the distance between the taps for pressure and vacuum, respectively. Brake horsepower was measured directly by an indicating wattmeter and corrected for motor efficiency from the motor-efficiency curve. Water horsepower and efficiency were computed from the foregoing data.

The velocity head is based on the net cross sectional area of discharge and suction pipes, respectively. The velocity of water in each instance was determined by this formula:

$$v=\frac{Q}{A}$$

in which v = velocity in feet per second, Q = flow in cubic feet per second, and A = net sectional area in square feet.

Velocity head was obtained from the formula:

$$h=\frac{v^2}{2g}$$

in which h = velocity head in feet, v = velocity in feet per second, and g = acceleration of gravity = 32.2.

The velocity-head difference is therefore equal to $\frac{v^2-v_1^2}{2g}$, in which v is the velocity of water in the discharge pipe, and v_1 is the velocity in the suction pipe. If the net area of the suction pipe at the point where the pipe is tapped for the measurement of the vacuum, is greater than that of the discharge pipe where it is tapped for the measurement of pressure, h is positive and is therefore additive to the total head composed of pressure, vacuum, and static heads. In the case of the pumps tested, the net area at the point of vacuum tap was less than the net area at the point of pressure tap, and hence the velocity of the water in the suction pipe was greater for a given volume than that in the discharge pipe. The velocity-head difference between discharge and suction pipes was therefore subtractive.

Diagrams based on the formula $h=\frac{v^2-v_1^2}{2g}$ have been prepared for each pump, as shown in Figures 3 to 8, inclusive, for the purpose of determining in the tests the amount of head to be added or subtracted due to the velocity of flow in the suction and discharge pipes.

In studying the diagrams, it is interesting to note the recovery of head due to velocity. Referring to Tables I and IV, comparing pumps Nos. 1 and 4, respectively, the recovery of head in pump No. 1 is shown to be 2.3 feet for a maximum flow of 1,321 gallons per minute, or 2.94 second-feet; while in pump No. 4 the recovery was 0.99 foot with a maximum flow of 1,466 gallons per minute, or 3.26 second-feet. Both pumps are 24 inches in diameter; No. 1 is 3-stage and No. 4 is 2-stage. They are of different makes, however, and the difference is due to the design, which gives a greater difference in area between discharge and suction in the case of No. 4 than No. 1. The recovery of head appears to be greater in the pumps of smaller diameter than in the larger pumps. For example, in pump No. 5, Table V, the velocity-head difference is 6.36 feet for a maximum flow of 1,092 gallons per minute, or 2.43 second-feet. This quantity is subtractive, indicating that there was no head recovered, but rather a loss of head, because the net area of discharge pipe at pressure tap was larger than the net area of the suction pipe at vacuum tap.

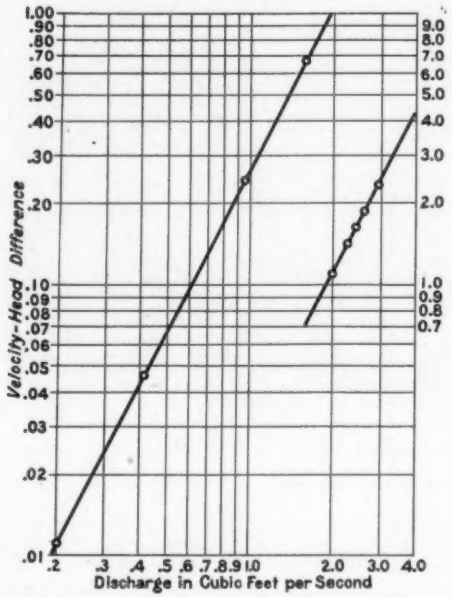


FIG. 3.—Velocity-head curve, pump No. 1, deep-well turbine pump, 3-stage, 24-inch diameter. Log curves show velocity-head difference between suction and discharge. Net area of suction where vacuum-gauge pipe is attached, 0.197 square foot. Net area of discharge where pressure-gauge pipe is attached, 0.343 square foot

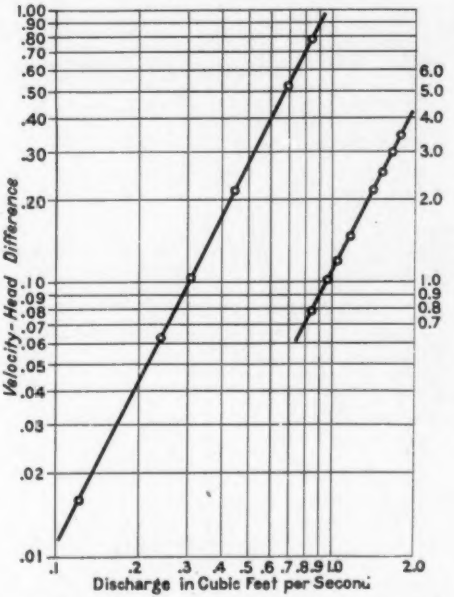


FIG. 4.—Velocity-head curve, pump No. 2, deep-well turbine pump, 5-stage, 14-inch diameter. Log curves show velocity difference between suction and discharge. Net area of suction where vacuum-gauge pipe is attached, 0.1085 square foot. Net area of discharge where pressure-gauge pipe is attached, 0.2670 square foot

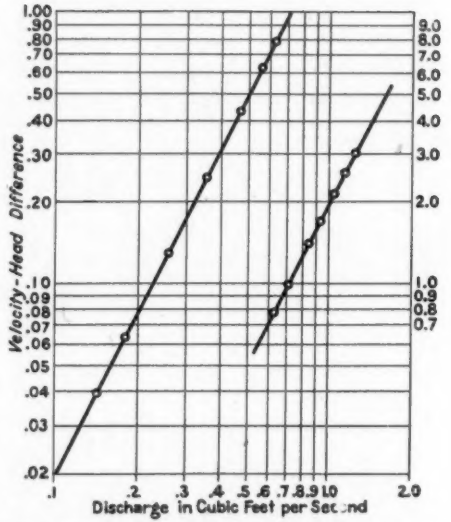


FIG. 5.—Velocity-head curve, pump No. 3, deep-well turbine pump, 7-stage, 12-inch diameter. Log curves show velocity-head difference between suction and discharge. Net area of suction where vacuum-gauge pipe is attached, 0.0841 square foot. Net area of discharge where pressure-gauge pipe is attached, 0.2670 square foot

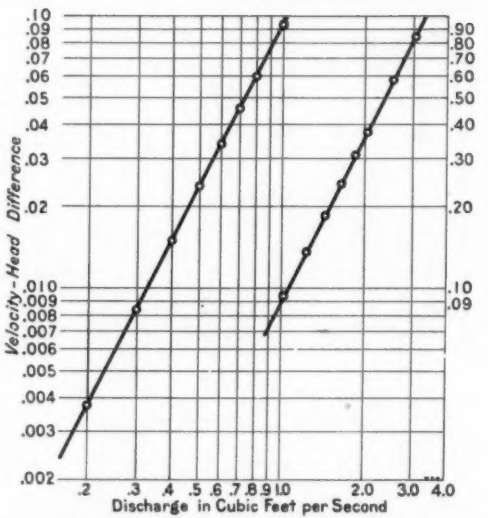


FIG. 6.—Velocity-head curve, pump No. 4, deep-well turbine pump, 2-stage, 24-inch diameter. Log curves show velocity-head difference between suction and discharge. Net area of suction where vacuum-gauge pipe is attached, 0.3078 square foot. Net area of discharge where pressure-gauge pipe is attached, 0.4700 square foot

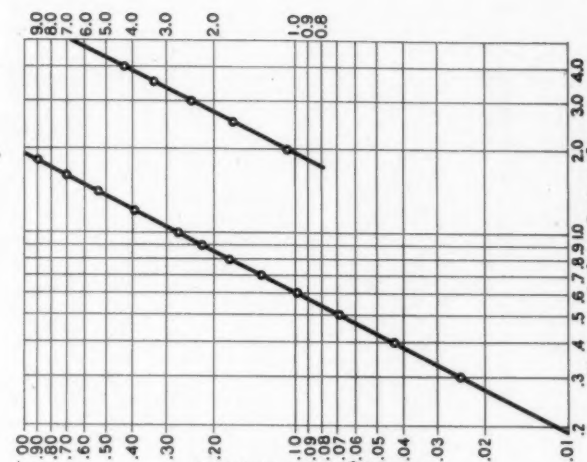


FIG. 8.—Velocity-head curve, pump No. 7, deep-well turbine pump, 4-stage, 17-inch diameter. Log curves show velocity-head difference between suction and discharge. Net area of suction where vacuum-gauge pipe is attached, 0.197 square foot. Net area of discharge where pressure-gauge pipe is attached, 0.349 square foot

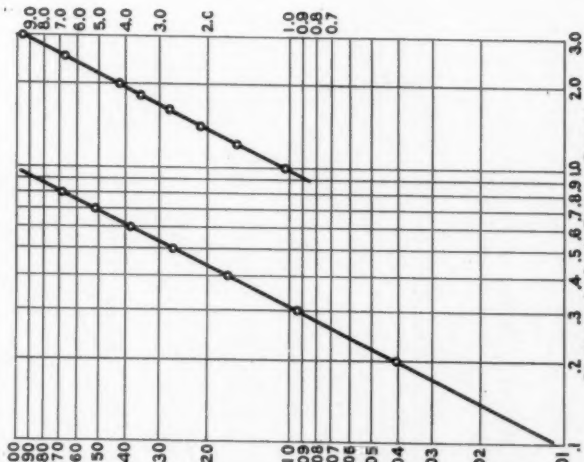


FIG. 7.—Velocity-head curve, pumps Nos. 5 and 6, deep-well turbine pump, 4-stage, 17-inch diameter. Log curves show velocity-head difference between suction and discharge. Net area of suction where vacuum-gauge is attached, 0.108 square foot. Net area of discharge where pressure-gauge pipe is attached, 0.249 square foot

TABLE I.—*Layne & Bowler 3-stage, 24-inch pump, No. 1, tested October 24 to December 4, 1914*

Average pump speed	Speed of pump	No. of run	Pressure head	Suction head	Static head	Velocity head	Total head	Brake horsepower	Discharge	Water horsepower	Efficiency
R. p. m.	*R. p. m.*		*Ft.*	*Ft.*	*Ft.*	*Ft.*	*Ft.*		*G p.m.*		*P. ct.*
	688	1	58.05	0.00	15.50	−0.00	73.55	7.62	0	0.00	0.0
	685	2	59.80	1.62	15.50	−.05	76.87	10.82	187	3.62	33.5
	686	3	53.40	4.15	15.50	−.24	72.81	14.34	423	7.76	54.1
	685	4	45.34	5.52	15.50	−.37	65.99	15.21	532	8.86	58.2
686	684	5	38.20	6.71	15.50	−.49	59.92	15.52	604	9.13	58.8
	685	6	32.11	7.75	15.50	−.60	54.76	15.78	672	9.29	58.9
	686	7	26.53	8.57	15.50	−.69	49.91	15.92	722	9.09	57.0
	687	8	20.45	9.48	15.50	−.78	44.65	16.12	772	8.70	53.9
	686	9	13.45	10.36	15.50	−.91	38.40	16.24	825	8.00	49.3
	686	10	6.12	11.17	15.50	−1.01	31.78	16.36	869	6.97	42.5
	772	1	75.20	.39	15.50	−.00	91.09	9.10	0	.00	.0
	765	2	78.70	2.03	15.50	−.04	96.19	12.86	178	4.32	33.5
	766	3	73.28	4.48	15.50	−.24	93.02	17.24	421	9.88	57.2
	759	4	68.90	4.46	15.50	−.29	88.57	18.16	468	10.45	57.5
	760	5	59.95	6.21	15.50	−.44	81.22	19.76	582	11.93	60.3
	760	6	49.75	7.91	15.50	−.63	72.53	20.72	690	12.63	60.9
762	761	7	43.80	8.92	15.50	−.72	67.50	20.38	741	12.62	61.8
	761	8	35.80	10.00	15.50	−.88	60.42	21.15	811	12.36	58.3
	760	9	28.60	11.01	15.50	−1.01	54.10	21.58	870	11.88	55.0
	758	10	21.74	11.78	15.50	−1.09	47.93	21.69	908	10.98	50.6
	762	11	16.71	12.37	15.50	−1.16	43.42	21.72	937	10.26	47.2
	760	12	11.19	12.94	15.50	−1.25	38.38	20.92	970	9.39	44.8
	761	13	6.80	13.47	15.50	−1.30	34.47	21.14	992	8.63	40.8
	837	1	92.85	.37	15.50	−.00	108.72	8.08	0	.00	.0
	834	2	96.80	2.51	15.50	−.09	114.72	17.88	266	7.70	43.0
	828	3	88.45	5.00	15.50	−.30	108.65	22.81	480	13.16	57.6
	826	4	78.90	6.62	15.50	−.46	100.56	23.70	593	15.02	63.4
	823	5	73.05	7.56	15.50	−.56	95.55	24.02	650	15.67	65.2
	822	6	62.40	9.02	15.50	−.74	86.18	24.69	745	16.20	65.5
	824	7	57.20	9.81	15.50	−.82	81.69	25.24	786	16.20	64.1
826	824	8	50.20	10.79	15.50	−.95	75.54	25.20	849	16.29	64.6
	824	9	42.90	11.75	15.50	−1.07	69.08	25.60	899	15.67	61.1
	821	10	36.50	12.57	15.50	−1.18	63.39	26.15	943	15.08	57.6
	822	11	28.90	13.41	15.50	−1.28	56.53	26.27	983	14.02	53.3
	823	12	22.40	14.04	15.50	−1.35	50.59	26.01	1,010	12.89	49.5
	827	13	16.27	14.86	15.50	−1.47	45.16	26.16	1,055	12.02	45.9
	826	14	11.59	14.88	15.50	−1.53	40.44	26.73	1,075	10.97	41.0
	828	15	7.01	15.46	15.50	−1.61	36.36	26.43	1,102	10.11	38.1
	912	1	112.65	.45	15.50	−.00	128.60	9.48	0	.00	.0
	903	2	115.51	3.16	15.50	−.14	134.03	22.26	318	10.75	48.3
	898	3	109.50	4.64	15.50	−.29	129.35	26.95	468	15.26	56.6
	896	4	101.85	6.11	15.50	−.44	123.02	28.50	577	17.91	62.8
	896	5	94.90	7.24	15.50	−.56	117.08	29.79	646	19.08	64.0
	897	6	85.80	8.49	15.50	−.70	109.19	30.64	727	20.03	65.3
	895	7	80.10	9.43	15.50	−.79	104.24	31.14	775	20.39	65.4
	895	8	72.00	10.57	15.50	−.95	97.12	32.03	846	20.73	64.7
898	896	9	66.97	11.33	15.50	−1.06	92.74	32.08	891	20.85	64.9
	895	10	57.80	12.49	15.50	−1.19	84.60	32.59	945	20.17	61.8
	893	11	50.50	13.21	15.50	−1.33	77.88	33.36	998	19.61	58.7
	896	12	45.16	13.92	15.50	−1.40	73.18	33.28	1,028	18.98	57.0
	899	13	38.53	14.86	15.50	−1.51	67.38	33.84	1,068	18.16	53.6
	897	14	31.40	15.53	15.50	−1.61	60.82	33.60	1,106	16.97	50.5
	897	15	26.34	16.12	15.50	−1.69	56.27	34.32	1,130	16.04	46.7
	898	16	18.97	16.94	15.50	−1.79	49.62	34.32	1,165	14.59	42.5
	895	17	13.34	17.46	15.50	−1.87	44.43	33.98	1,190	13.34	39.2
	897	18	7.56	17.90	15.50	−1.93	39.03	33.73	1,210	11.91	35.3
	1,026	1	147.50	.06	[a] 17.54	−.00	165.04	18.12	0	.00	.0
	1,026	2	148.70	.73	17.54	−.01	166.96	21.58	94	3.96	18.3
	1,020	3	151.18	1.55	17.54	−.05	170.22	25.51	192	8.24	32.3
	1,025	4	152.75	2.54	17.54	−.11	172.72	29.09	282	12.29	42.3
	1,018	5	148.20	3.50	17.54	−.18	169.06	32.29	365	15.57	48.2
	1,009	6	142.85	5.14	17.54	−.32	165.21	36.07	493	20.55	56.9
	1,012	7	135.83	7.04	17.54	−.51	159.90	40.28	624	25.18	62.5
	1,009	8	125.00	8.48	17.54	−.67	150.35	42.04	710	26.94	64.1
	1,011	9	119.33	9.49	15.50	−.78	143.54	42.97	766	27.75	64.5
	1,009	10	115.37	9.81	15.50	−.87	139.81	45.02	807	28.47	63.2
	1,009	11	106.68	11.10	15.50	−1.00	132.28	45.74	866	28.91	63.2
1,011	1,009	12	100.12	11.92	15.50	−1.12	126.42	46.67	921	29.38	62.9
	1,012	13	91.44	13.39	15.50	−1.29	119.04	48.10	986	29.62	61.6
	1,010	14	80.80	14.69	15.50	−1.46	109.53	48.22	1,049	28.99	60.0
	1,009	15	70.30	15.93	15.50	−1.61	100.12	48.62	1,100	27.79	57.1
	1,008	16	61.93	16.69	15.50	−1.76	92.36	48.80	1,150	26.80	54.9
	1,008	17	57.06	17.37	15.50	−1.82	88.11	48.98	1,169	25.98	53.0
	1,006	18	49.16	18.16	15.50	−1.95	80.87	48.34	1,202	24.54	50.7
	1,004	19	37.86	19.24	15.50	−2.05	70.55	48.22	1,246	22.18	45.9
	1,003	20	31.41	19.78	15.50	−2.13	64.56	47.46	1,274	20.75	43.7
	1,003	21	21.47	20.51	15.50	−2.18	55.30	47.39	1,287	17.96	37.9
	999	22	8.48	21.19	15.50	−2.31	42.86	46.82	1,321	14.29	30.5

[a] Static head of mercury column=17.54 feet. Static head of pressure gauge=15.50 feet.

TABLE II.—*Layne & Bowler 5-stage, 14-inch pump, No. 2, tested March 22 to April 15, 1915*

Average pump speed	Speed of pump	No. of run	Pressure head	Suction head	Static head	Velocity head	Total head	Brake horsepower	Discharge	Water horsepower	Efficiency
R. p. m.	*R. p. m.*		*Ft.*	*Ft.*	*Ft.*	*Ft.*	*Ft.*		*G.p.m.*		*P. ct.*
	890	1	83.00	1.13	13.58	−0.00	97.71	6.46	0	0.00	0.0
	887	2	88.90	1.47	13.58	−.02	103.93	7.58	55	1.44	19.0
	886	3	86.67	2.49	13.58	−.14	102.60	9.88	162	4.19	42.4
	882	4	81.85	3.28	13.58	−.30	98.41	11.76	235	5.83	49.6
	882	5	75.30	4.24	13.58	−.50	92.62	12.45	305	7.13	57.3
	878	6	71.88	4.50	13.58	−.55	89.41	13.41	319	7.19	53.6
	878	7	62.20	5.37	13.58	−.79	80.36	14.11	382	7.74	54.8
	878	8	55.10	6.04	13.58	−.95	73.77	13.82	421	7.83	56.7
880	878	9	47.80	6.55	13.58	−1.12	66.81	14.31	456	7.68	53.7
	879	10	44.25	6.86	13.58	−1.19	63.50	13.76	472	7.56	54.9
	878	11	37.60	7.40	13.58	−1.33	57.25	14.27	498	7.19	50.3
	880	12	31.50	7.80	13.58	−1.47	51.41	14.63	520	6.74	46.1
	881	13	26.00	8.13	13.58	−1.57	46.14	14.04	538	6.26	44.6
	876	14	19.40	8.64	13.58	−1.69	39.93	14.06	559	5.63	40.0
	876	15	13.61	8.89	13.58	−1.80	34.28	13.83	574	4.96	35.9
	873	16	6.78	9.27	13.58	−1.90	27.73	13.87	595	4.16	30.0
	988	1	106.50	1.41	13.58	−.00	121.49	6.39	0	.00	.0
	979	2	111.60	2.15	13.58	−.06	127.27	11.74	108	3.47	29.6
	974	3	108.04	3.17	13.58	−.22	124.57	14.02	202	6.35	45.2
	981	4	99.69	4.58	13.58	−.52	117.33	15.66	312	9.25	59.1
	977	5	89.85	5.54	13.58	−.78	108.19	17.28	379	10.35	59.8
	973	6	77.69	5.93	13.58	−.96	96.24	17.63	422	10.25	58.1
	976	7	68.70	6.84	13.58	−1.19	87.93	17.78	468	10.38	58.3
	976	8	62.30	7.40	13.58	−1.35	81.93	16.88	500	10.34	61.1
978	979	9	56.20	7.91	13.58	−1.54	76.15	17.07	531	10.20	59.7
	976	10	48.90	8.48	13.58	−1.68	69.28	17.14	556	9.72	56.6
	972	11	43.25	8.87	13.58	−1.80	63.90	18.64	574	9.26	49.6
	974	12	37.45	9.38	13.58	−1.91	58.50	18.67	595	8.78	47.0
	976	13	31.90	9.77	13.58	−2.05	53.20	16.62	614	8.24	49.5
	982	14	26.00	10.17	13.58	−2.16	47.59	18.19	630	7.56	41.6
	980	15	18.64	10.65	13.58	−2.32	40.55	17.56	654	6.69	38.0
	982	16	12.94	10.96	13.58	−2.40	35.08	17.56	665	5.88	33.5
	980	17	6.55	11.19	13.58	−2.51	28.81	18.07	679	4.93	27.3
	1,078	1	128.30	1.47	13.58	−.00	143.35	8.60	0	.00	.0
	1,075	2	137.85	2.20	13.58	−.05	153.58	12.78	97	3.75	29.3
	1,072	3	136.00	2.26	13.58	−.14	151.70	12.96	164	6.27	48.4
	1,068	4	128.20	3.73	13.58	−.45	145.06	17.15	290	10.62	61.8
	1,064	5	120.80	4.53	13.58	−.67	138.24	18.16	350	12.21	67.2
	1,064	6	112.15	5.45	13.58	−.90	130.28	20.61	406	13.35	64.7
	1,064	7	103.40	6.16	13.58	−1.06	122.08	20.74	445	13.71	66.0
	1,062	8	95.70	6.81	13.58	−1.24	115.12	21.19	477	13.85	65.3
	1,066	9	87.10	7.52	13.58	−1.39	106.81	22.62	508	13.69	60.4
1,066	1,062	10	78.85	8.14	13.58	−1.62	98.95	23.25	544	13.58	58.4
	1,064	11	68.35	8.82	13.58	−1.82	88.93	23.49	579	12.99	55.3
	1,065	12	60.20	9.44	13.58	−2.02	81.20	23.32	609	12.48	53.4
	1,058	13	50.70	9.77	13.58	−2.11	71.94	23.20	622	11.29	48.6
	1,062	14	42.80	10.28	13.58	−2.27	64.39	23.14	649	10.55	45.5
	1,064	15	32.90	10.96	13.58	−2.46	54.98	23.08	672	9.32	40.3
	1,065	16	27.20	11.30	13.58	−2.62	49.46	22.96	693	8.65	37.6
	1,064	17	20.39	11.81	13.58	−2.72	43.06	22.51	706	7.67	34.0
	1,068	18	7.07	12.45	13.58	−2.98	30.12	22.56	740	5.62	24.9
	1,174	1	161.60	1.30	* 16.58	−.00	179.48	11.40	0	.00	.0
	1,171	2	164.40	1.92	16.58	−.03	182.87	12.61	79	3.65	28.9
	1,164	3	156.50	3.64	16.58	−.34	176.38	21.48	250	11.11	51.7
	1,162	4	151.00	4.75	16.58	−.58	171.75	23.35	326	14.13	60.5
	1,164	5	142.80	5.33	16.58	−.81	163.90	26.07	387	16.00	61.3
	1,162	6	134.20	6.16	16.58	−1.03	155.91	26.68	434	17.08	63.9
	1,160	7	126.96	6.86	13.58	−1.21	146.19	26.97	473	17.45	64.6
	1,162	8	119.45	7.48	13.58	−1.37	139.14	27.55	505	17.73	64.3
	1,159	9	111.55	8.14	13.58	−1.53	131.74	28.29	533	17.72	62.7
	1,160	10	103.40	8.81	13.58	−1.75	124.04	28.68	565	17.68	61.6
	1,157	11	94.00	9.47	13.58	−1.93	115.12	28.73	596	17.32	60.2
1,161	1,160	12	88.05	10.03	13.58	−2.12	109.54	29.07	624	17.25	59.3
	1,161	13	81.60	10.45	13.58	−2.22	103.41	28.98	641	16.72	57.6
	1,161	14	74.50	10.85	13.58	−2.40	96.53	29.06	665	16.20	55.7
	1,164	15	66.91	11.27	13.58	−2.51	89.25	27.86	682	15.36	55.0
	1,160	16	58.89	11.76	13.58	−2.67	81.56	28.83	700	14.41	49.9
	1,160	17	53.90	12.03	13.58	−2.75	76.76	28.02	709	13.73	49.0
	1,158	18	47.15	12.31	13.58	−2.83	70.21	28.00	722	12.79	45.6
	1,156	19	39.80	12.65	13.58	−2.91	63.12	28.56	734	11.69	40.9
	1,158	20	33.34	13.11	13.58	−3.10	56.93	28.80	754	10.82	37.6
	1,158	21	27.10	13.44	13.58	−3.16	50.96	28.68	762	9.80	34.1
	1,158	22	28.80	12.89	13.58	−3.11	52.16	29.08	754	9.92	34.1
	1,153	23	21.90	13.32	13.58	−3.21	45.59	28.93	768	8.83	30.5
	1,158	24	15.50	13.72	13.58	−3.28	39.52	29.03	776	7.74	26.6

* Static head for pressure gauge=16.58. Static head for mercury column=13.58.

TABLE III.—*Layne & Bowler 7-stage, 12-inch pump, No. 3, tested January 27 to February 1, 1915*

Average pump speed.	Speed of pump	No. of run	Pressure head	Suction head	Static head	Velocity head	Total head	Brake horsepower	Discharge	Water horsepower	Efficiency
R. p. m.	*R. p. m.*		*Ft.*	*Ft.*	*Ft.*	*Ft.*	*Ft.*		*G. p. m.*		*Per ct.*
	990	1	87.00	0.90	14.15	−0.00	102.05	4.96	0	0.00	0.0
	990	2	90.20	1.53	14.15	−.07	105.81	7.53	85	2.26	30.0
	990	3	89.49	1.41	14.15	−.09	104.96	6.35	94	2.49	39.2
	984	4	80.80	2.06	14.15	−.24	96.77	7.73	157	3.83	49.5
	986	5	73.70	2.66	14.15	−.41	90.10	7.71	205	4.66	60.4
	989	6	65.50	3.33	14.15	−.63	82.35	8.48	253	5.25	61.9
987	988	7	59.95	3.73	14.15	−.77	77.06	8.07	279	5.42	67.2
	985	8	53.35	4.18	14.15	−.93	70.75	8.50	306	5.46	64.2
	988	9	46.60	4.63	14.15	−1.10	64.28	7.95	334	5.41	68.1
	988	10	39.50	5.14	14.15	−1.25	57.54	7.91	359	5.21	65.9
	988	11	33.65	5.48	14.15	−1.42	51.86	7.75	381	4.98	64.3
	986	12	25.55	5.93	14.15	−1.60	44.03	7.52	404	4.48	59.6
	982	13	5.42	7.06	14.15	−2.07	24.56	6.96	461	2.85	40.9
	1,028	1	98.30	0.51	14.15	−.00	112.96	6.76	0	0.00	0.0
	1,030	2	101.10	1.13	14.15	−.06	116.32	9.40	81	2.37	25.2
	1,032	3	93.60	1.64	14.15	−.18	109.21	10.16	136	3.75	36.8
	1,031	4	83.10	2.49	14.15	−.43	99.31	11.70	209	5.23	44.7
	1,030	5	60.65	4.17	14.15	−.97	78.00	10.46	313	6.16	58.8
1,030	1,029	6	45.35	5.13	14.15	−1.34	63.29	10.04	370	5.91	58.8
	1,031	7	37.30	5.65	14.15	−1.55	55.55	9.82	397	5.56	56.6
	1,031	8	31.41	5.87	14.15	−1.69	49.74	10.52	416	5.22	49.5
	1,030	9	23.56	6.55	14.15	−1.86	42.40	9.47	437	4.67	49.3
	1,029	10	14.92	7.01	14.15	−2.10	33.98	9.49	464	3.97	41.8
	1,032	11	5.71	7.57	14.15	−2.35	25.08	8.82	488	3.08	34.9
	1,078	1	108.17	0.85	14.15	−.00	123.17	7.35	0	.00	.0
	1,078	2	112.60	1.24	14.15	−.04	127.95	8.46	63	2.03	24.0
	1,070	3	100.80	2.20	14.15	−.26	116.89	11.27	162	4.77	42.3
	1,073	4	90.80	2.77	14.15	−.47	107.25	12.94	220	5.95	46.0
	1,066	5	81.60	3.50	14.15	−.70	98.55	13.16	267	6.64	50.4
	1,068	6	70.40	4.21	14.15	−.98	87.78	13.36	315	6.97	52.2
	1,071	7	63.20	4.77	14.15	−1.16	80.96	13.08	343	7.00	53.5
1,073	1,073	8	56.10	5.25	14.15	−1.34	74.16	13.30	370	6.92	52.0
	1,072	9	49.20	5.71	14.15	−1.52	67.54	13.13	395	6.73	51.2
	1,073	10	42.40	6.10	14.15	−1.69	60.96	12.43	416	6.40	51.4
	1,072	11	34.85	6.55	14.15	−1.85	53.70	10.72	435	5.89	54.9
	1,073	12	26.34	7.06	14.15	−2.07	45.48	10.26	461	5.29	51.5
	1,076	13	13.30	7.85	14.15	−2.40	32.90	9.06	490	4.06	44.8
	1,073	14	5.76	8.28	14.15	−2.67	25.52	8.81	518	3.33	37.8
	1,171	1	130.25	0.56	14.15	−.00	144.96	7.64	0	.00	.0
	1,168	2	131.85	1.47	14.15	−.13	147.34	12.10	114	4.23	35.0
	1,170	3	123.70	2.12	14.15	−.29	139.68	11.89	174	6.13	51.6
	1,170	4	115.65	2.66	14.15	−.46	132.00	12.43	218	7.26	58.3
	1,164	5	106.65	3.33	14.15	−.67	123.46	13.42	261	8.13	60.5
	1,166	6	97.05	3.95	14.15	−.90	114.25	15.82	302	8.70	54.9
	1,166	7	85.15	4.69	14.15	−1.17	102.82	15.74	345	8.95	56.8
	1,162	8	77.60	5.20	14.15	−1.35	95.60	16.16	372	8.97	55.5
	1,164	9	70.23	5.65	14.15	−1.50	88.53	16.43	391	8.73	53.1
1,168	1,169	10	64.40	6.10	14.15	−1.69	82.96	16.02	416	8.70	54.3
	1,167	11	55.70	6.55	14.15	−1.90	74.50	15.68	440	8.27	52.7
	1,165	12	48.80	6.95	14.15	−2.08	67.82	15.74	461	7.89	50.1
	1,172	13	47.50	6.95	14.15	−2.16	66.44	16.22	468	7.84	48.3
	1,172	14	40.70	7.40	14.15	−2.35	59.90	15.66	490	7.40	47.2
	1,172	15	35.20	7.74	14.15	−2.49	54.60	15.32	505	6.95	45.4
	1,168	16	29.94	8.02	14.15	−2.65	49.46	15.40	515	6.42	41.7
	1,168	17	22.60	8.48	14.15	−2.82	42.41	14.79	533	5.70	38.5
	1,171	18	16.05	8.87	14.15	−2.97	36.10	14.76	551	5.02	33.9
	1,172	19	7.23	9.38	14.15	−3.16	27.60	14.12	569	3.96	28.0

TABLE IV.—*American 2-stage, 24-inch pump, No. 4, tested January 29, March 9, March 11, 1916*

Average pump speed	Speed of pump	No. of run	Pressure head	Suction head	Static head	Velocity head	Total head	Brake horsepower	Discharge	Water horsepower	Efficiency
R. p. m.	*R. p. m.*		*Ft.*	*Ft.*	*Ft.*	*Ft.*	*Ft.*		*G. p. m.*		*P. ct.*
	898	1	93.37	0.00	14.98	−0.00	108.35	12.92	0	0.00	0.0
	893	2	87.58	.00	14.98	−.01	102.55	17.82	142	3.67	20.5
	892	3	78.14	1.07	14.98	−.04	94.15	22.04	313	7.43	33.7
	889	4	70.18	3.05	14.98	−.11	88.10	24.08	492	10.94	45.4
	888	5	60.46	5.22	14.98	−.20	80.46	28.11	657	13.36	47.5
	885	6	51.64	7.48	14.98	−.34	73.76	29.95	807	15.06	50.3
890	885	7	43.96	9.74	14.98	−.40	68.28	30.19	936	16.13	53.5
	888	8	35.14	12.23	14.98	−.53	61.82	30.39	1,084	16.92	55.6
	889	9	27.12	14.69	14.98	−.66	56.13	30.87	1,206	17.09	55.3
	889	10	14.12	17.09	14.98	−.82	45.37	30.07	1,332	15.26	50.6
	889	11	12.03	19.29	14.98	−.92	45.38	27.00	1,408	16.13	59.6
	892	12	73.96	2.32	14.98	−.07	91.19	21.19	391	9.00	42.5
	888	13	63.22	4.80	14.98	−.16	82.84	26.47	594	12.42	46.8
	936	1	105.09	.00	14.98	−.00	120.07	17.24	0	.00	.0
	934	2	96.95	.14	14.98	−.02	112.05	20.93	181	5.11	24.4
	932	3	87.58	1.31	14.98	−.06	103.81	24.49	358	9.38	38.3
	929	4	78.80	2.77	14.98	−.11	96.44	26.38	487	11.85	44.9
	929	5	69.05	4.89	14.98	−.20	88.72	29.44	654	14.65	49.7
928	927	6	60.22	8.13	14.98	−.29	83.04	32.18	792	16.59	51.6
	926	7	51.70	9.46	14.98	−.40	75.74	33.96	933	17.84	52.5
	926	8	44.24	11.64	14.98	−.51	70.35	34.60	1,058	18.79	54.2
	924	9	35.48	14.18	14.98	−.64	64.00	35.20	1,183	19.11	54.2
	923	10	27.01	16.56	14.98	−.77	57.78	35.67	1,300	18.96	53.1
	926	11	11.75	20.28	14.98	−.98	46.03	33.57	1,465	17.03	50.7
	995	1	120.97	.00	14.98	−.00	135.95	19.11	0	.00	.0
	993	2	114.46	.00	14.98	−.01	129.43	23.66	131	4.30	18.2
	993	3	109.27	.48	14.98	−.03	124.70	26.07	241	7.57	29.0
	987	4	102.55	.99	14.98	−.04	118.48	28.97	305	9.11	31.4
	990	5	99.89	2.15	14.98	−.08	116.94	31.58	403	11.88	37.6
	990	6	91.98	3.05	14.98	−.11	109.90	33.23	487	13.51	40.6
987	987	7	84.09	5.08	14.98	−.19	103.96	36.50	640	16.80	46.0
	984	8	73.96	7.03	14.98	−.28	95.69	38.46	778	18.79	48.8
	984	9	65.94	9.35	14.98	−.37	89.90	40.43	899	20.40	50.4
	982	10	62.37	10.38	14.98	−.43	87.30	40.84	967	21.31	52.1
	984	11	53.56	12.60	14.98	−.54	80.60	42.05	1,097	22.32	53.0
	982	12	45.19	14.95	14.98	−.66	74.46	42.44	1,199	22.54	53.1
	981	13	36.89	17.40	14.98	−.80	68.47	42.10	1,321	22.83	54.2
	985	14	13.05	20.31	14.98	−1.09	47.25	39.50	1,468	17.55	44.4
	1,090	1	143.77	.00	18.56	−.00	162.33	22.82	0	.00	.0
	1,084	2	136.50	.00	14.98	−.01	151.47	29.20	150	5.73	19.6
	1,080	3	129.66	.45	14.98	−.03	145.06	33.73	267	9.76	28.9
	1,075	4	118.65	2.09	14.98	−.08	135.64	39.04	416	14.24	36.4
	1,073	5	111.19	3.73	14.98	−.14	129.76	42.78	551	18.05	42.1
	1,068	6	101.58	5.82	14.98	−.22	122.16	45.70	699	21.56	47.1
1,071	1,069	7	93.00	7.63	14.98	−.30	115.31	48.27	818	23.81	49.3
	1,068	8	84.52	9.72	14.98	−.40	108.82	50.13	933	25.63	51.1
	1,065	9	83.73	9.38	14.98	−.40	107.69	50.68	940	25.56	50.3
	1,063	10	76.05	11.45	14.98	−.50	101.98	51.42	1,046	26.93	52.3
	1,064	11	66.44	13.90	14.98	−.64	94.68	52.75	1,176	28.11	53.2
	1,062	12	58.37	15.93	14.98	−.77	88.54	52.67	1,280	28.61	54.2
	1,061	13	50.51	17.79	14.98	−.90	82.38	52.51	1,401	29.17	55.4
	1,066	14	41.24	19.61	14.98	−.99	74.87	51.88	1,466	27.11	53.3

TABLE V.—*American 4-stage, 17-inch pump, No. 5, tested March 20 and March 28, 1916*

Average pump speed	Speed of pump	No. of run	Pressure head	Suction head	Static head	Velocity head	Total head	Brake horsepower	Discharge	Water horsepower	Efficiency
R. p. m.	*R. p. m.*		*Ft.*	*Ft.*	*Ft.*	*Ft.*	*Ft.*		*G.p.m.*		*P. ct.*
	865	1	105.66	1.46	11.59	−0.00	118.71	2.60	0	0.00	0.0
	860	2	90.96	2.71	11.59	−.10	105.16	11.30	137	3.63	32.1
	856	3	81.65	3.73	11.59	−.27	96.70	14.02	225	5.48	39.2
	854	4	74.23	4.78	11.59	−.50	90.10	16.74	309	7.02	41.9
	853	5	64.07	6.98	11.59	−1.01	81.63	18.57	435	8.95	48.2
857	853	6	56.96	8.50	11.59	−1.36	75.69	18.04	505	9.65	53.4
	854	7	47.06	10.48	11.59	−1.86	67.27	18.64	590	10.02	53.8
	855	8	38.25	12.29	11.59	−2.30	59.83	17.65	657	9.91	56.2
	856	9	29.15	14.46	11.59	−2.86	52.34	15.97	732	9.66	60.4
	857	10	20.57	16.58	11.59	−3.34	45.40	16.39	792	9.06	55.3
	858	11	8.23	20.28	11.59	−4.32	35.78	14.91	900	8.12	54.5
	945	1	123.85	1.64	11.59	−.00	137.08	4.07	0	.00	.0
	940	2	111.47	2.85	11.59	−.12	125.79	9.93	149	4.72	47.5
	934	3	101.08	3.98	11.59	−.28	116.37	15.51	231	6.77	43.6
	933	4	92.20	5.11	11.59	−.55	108.35	20.98	325	8.87	42.3
934	932	5	82.89	6.92	11.59	−1.10	100.30	23.01	455	11.50	49.9
	930	6	73.18	8.95	11.59	−1.46	92.26	24.17	523	12.16	50.3
	931	7	62.99	10.85	11.59	−1.90	83.53	24.05	596	12.55	52.2
	933	8	47.57	14.12	11.59	−2.73	70.55	23.66	716	12.73	53.8
	933	9	25.14	19.52	11.59	−4.17	52.08	22.43	884	11.62	51.7
	935	10	8.14	24.66	11.59	−5.47	38.92	16.15	1,012	9.93	61.5
	1,090	1	183.08	2.04	14.57	−.00	199.69	16.55	0	.00	.0
	1,085	2	159.44	2.68	14.57	−.06	176.63	18.97	108	4.81	25.4
	1,081	3	145.15	3.39	14.57	−.21	162.90	24.04	201	8.25	34.3
	1,081	4	138.14	4.55	11.59	−.43	153.85	27.98	286	11.09	39.6
	1,078	5	124.20	6.35	11.59	−.91	141.23	33.57	411	14.63	43.6
	1,076	6	111.70	8.76	11.59	−1.59	130.46	36.14	536	17.63	48.8
1,079	1,074	7	97.63	11.48	11.59	−2.21	118.49	37.31	644	19.26	51.5
	1,075	8	83.05	14.24	11.59	−2.95	105.93	37.78	744	19.89	52.6
	1,075	9	68.93	17.12	11.59	−3.67	93.97	37.31	829	19.66	52.6
	1,076	10	56.16	20.02	11.59	−4.35	83.42	36.11	903	19.01	52.6
	1,078	11	43.56	23.11	11.59	−5.12	73.14	34.50	980	18.09	52.3
	1,076	12	30.17	26.72	11.59	−6.13	62.35	33.22	1,071	16.85	50.7
	1,077	13	8.70	29.04	11.59	−6.55	42.78	32.33	1,108	11.95	36.9
	1,186	1	220.83	2.43	14.57	−.00	237.83	14.28	0	.00	.0
	1,178	2	197.67	2.88	14.57	−.04	215.08	24.16	94	5.09	21.1
	1,177	3	184.32	3.81	14.57	−.14	202.56	28.26	165	8.42	29.8
	1,176	4	171.76	4.24	14.57	−.30	190.27	32.42	241	11.57	35.7
	1,171	5	155.24	5.78	14.57	−.72	174.87	38.62	366	16.15	41.8
1,169	1,166	6	135.95	8.74	14.57	−1.52	157.74	43.57	534	21.26	48.7
	1,164	7	119.90	12.17	11.59	−2.38	141.28	45.70	668	23.81	52.0
	1,158	8	101.46	15.48	11.59	−3.20	125.33	46.08	775	24.51	53.1
	1,158	9	84.01	19.07	11.59	−4.12	110.55	45.67	878	24.49	53.6
	1,163	10	65.31	23.36	11.59	−5.22	95.04	44.63	989	23.72	53.2
	1,165	11	46.08	27.68	11.59	−6.36	78.99	43.89	1,092	21.77	49.5

In order to facilitate the plotting of the performance curves, characteristic curves similar to those shown in Figure 9 were plotted for each average speed of pump. The efficiency, total head, speed, brake horsepower, and water horsepower were plotted as ordinates, and the corresponding discharges in gallons per minute were plotted as abscissas from data given in Tables 1 to 7, inclusive. From these characteristic curves, the performance curves were produced by plotting the capacity-head curves for the various speeds of each pump on the same sheet. Points of equal efficiency taken from the characteristic curves were connected so as to form iso-efficiency curves. In the same manner points of equal brake horsepower were taken from the characteristic curves and connected to form curves showing points of equal brake horsepower.

TABLE VI.—*American 2-stage, 17-inch pump, No. 6*

Average pump speed	Speed of pump	No. of run	Pressure head	Suction head	Static head	Velocity head	Total head	Brake horsepower	Discharge	Water horsepower	Efficiency
R. p. m.	*R. p. m.*		*Ft.*	*Ft.*	*Ft.*	*Ft.*	*Ft.*		*G. p. m.*		*P. ct.*
	768	1	36.16	0.56	11.62	−0.00	48.34	5.51	0	0.00	0.0
	766	2	32.09	1.24	11.62	−.02	44.93	5.82	53	.60	10.3
	768	3	28.70	1.69	11.62	−.07	41.94	6.13	112	1.18	19.2
768	767	4	22.82	3.05	11.62	−.29	37.20	7.16	236	2.21	30.9
	769	5	17.74	4.86	11.62	−.66	33.56	7.86	350	2.96	37.7
	770	6	13.11	6.78	11.62	−1.05	30.46	8.31	446	3.43	41.3
	767	7	7.23	9.72	11.62	−1.76	26.81	8.22	572	3.87	47.1
	862	1	49.04	.23	11.62	−.00	60.89	2.24	0	.00	.0
	856	2	42.94	1.18	11.62	−.03	55.71	4.92	74	1.04	21.2
	854	3	39.66	1.69	11.62	−.06	52.91	6.15	122	1.62	26.3
	855	4	35.59	2.49	11.62	−.21	49.49	6.40	198	2.47	38.6
854	852	5	28.76	4.26	11.62	−.58	44.06	8.46	333	3.70	43.7
	851	6	22.15	6.67	11.62	−1.15	39.29	10.60	464	4.60	43.4
	851	7	18.08	8.19	11.62	−1.52	36.37	10.09	533	4.89	48.5
	852	8	13.90	8.83	11.62	−1.92	32.43	10.26	601	4.93	48.0
	851	9	7.06	13.31	11.62	−2.66	29.33	10.44	711	5.26	50.3
	935	1	61.06	.28	11.62	−.00	72.96	6.89	0	.00	.0
	938	2	55.48	1.13	11.62	−.02	68.21	5.90	53	.91	15.4
	937	3	50.85	1.86	11.62	−.07	64.26	8.40	115	1.86	22.1
	936	4	46.49	2.20	11.62	−.18	60.13	8.32	186	2.82	33.9
934	932	5	39.55	3.84	11.62	−.49	54.52	10.45	306	4.20	40.2
	932	6	31.98	6.41	11.62	−1.05	48.96	12.15	446	5.50	45.3
	930	7	27.57	8.05	11.62	−1.44	45.80	12.97	518	5.98	46.1
	931	8	20.45	10.73	11.62	−2.10	40.70	13.39	630	6.46	48.2
	932	9	14.06	13.56	11.62	−2.76	36.48	13.39	724	6.66	49.7
	932	10	7.32	16.72	11.62	−3.57	32.09	13.45	825	6.68	49.6
	1,004	1	69.21	.45	11.62	−.00	81.28	5.90	0	.00	.0
	999	2	62.72	1.58	11.62	−.02	75.90	6.65	71	1.36	20.5
	995	3	56.39	2.20	11.62	−.12	70.09	9.27	153	2.70	29.1
	992	4	48.31	3.78	11.62	−.41	63.30	11.77	280	4.47	38.0
995	990	5	39.60	6.78	11.62	−1.12	56.88	14.10	458	6.57	46.6
	992	6	30.74	10.40	11.62	−1.81	50.95	15.15	582	7.48	49.4
	993	7	22.83	13.73	11.62	−2.81	45.37	15.18	727	8.32	54.8
	993	8	10.17	19.16	11.62	−4.32	36.63	14.77	902	8.34	56.4
	1,095	1	84.18	.59	11.62	−.00	96.39	6.71	0	.00	.0
	1,093	2	75.69	2.20	11.62	−.04	89.38	10.80	87	1.96	18.1
	1,092	3	66.85	2.82	11.62	−.18	81.11	11.35	186	3.80	33.5
	1,093	4	59.72	4.63	11.62	−.48	75.49	11.68	300	5.70	48.8
	1,091	5	50.85	7.03	11.62	−1.04	68.46	17.22	444	7.66	44.5
	1,090	6	41.92	10.17	11.62	−1.82	61.87	19.11	584	9.14	47.8
1,086	1,080	8	44.41	9.04	11.62	−1.55	63.52	19.15	541	8.66	45.2
	1,080	9	37.23	11.64	11.62	−2.17	58.32	19.96	641	9.42	47.2
	1,082	10	36.61	12.20	11.62	−2.50	57.93	20.13	685	10.00	49.7
	1,082	11	29.27	15.03	11.62	−3.20	52.72	20.23	775	10.30	50.9
	1,082	12	22.31	18.08	11.62	−3.98	48.03	20.22	864	10.46	51.7
	1,082	13	16.84	20.28	11.62	−4.51	44.23	19.81	921	10.28	51.9
	1,082	14	8.19	24.35	11.62	−5.55	38.61	19.60	1,023	9.95	50.8
	1,191	1	103.55	.49	11.62	−.00	115.66	2.40	0	.00	.0
	1,190	2	94.68	1.69	11.62	−.02	107.97	8.82	62	1.69	19.2
	1,189	3	90.46	2.46	11.62	−.08	104.46	11.73	124	3.26	27.8
	1,189	4	84.18	2.82	11.62	−.14	98.48	12.43	166	4.12	33.1
	1,188	5	79.16	3.28	11.62	−.30	93.76	16.48	237	5.60	34.0
	1,183	6	72.07	4.89	11.62	−.54	88.04	19.09	319	7.09	37.1
	1,186	7	68.93	5.31	11.62	−.69	85.17	18.08	361	7.75	42.9
1,184	1,182	8	64.64	6.33	11.62	−1.00	81.59	22.05	444	9.13	41.4
	1,182	9	59.78	7.91	11.62	−1.43	77.88	22.63	518	10.17	44.9
	1,180	10	54.80	9.83	11.62	−1.87	74.38	23.66	595	11.17	46.9
	1,180	11	44.24	13.56	11.62	−2.81	66.61	25.23	732	12.29	48.7
	1,176	12	37.52	16.10	11.62	−3.46	61.78	25.61	804	12.57	49.0
	1,180	13	30.51	19.89	11.62	−4.25	57.77	26.20	896	13.06	49.8
	1,180	14	22.31	24.01	11.62	−5.40	52.54	25.58	1,008	13.39	52.3
	1,178	15	13.00	27.37	11.62	−6.14	45.85	25.22	1,074	12.41	49.2

TABLE VII.—*Byron-Jackson 4-stage, 17-inch pump, No. 7*

Average pump speed	Speed of pump	No. of run	Pressure head	Suction head	Static head	Velocity head	Total head	Brake horsepower	Discharge	Water horsepower	Efficiency
R. p. m.	*R. p. m.*		*Ft.*	*Ft.*	*Ft.*	*Ft.*	*Ft.*		*G. p. m.*		*P. ct.*
	878	1	53.79	0.00	12.26	−0.00	66.05	9.09	0	0.00	0.0
	879	2	58.76	1.13	12.26	−.02	72.13	9.70	109	1.98	20.4
	880	3	58.99	1.58	12.26	−.07	72.76	12.13	230	4.22	34.8
879	879	4	54.24	3.16	12.26	−.25	69.41	14.46	437	7.64	52.8
	880	5	48.15	4.97	12.26	−.42	64.96	15.00	561	9.18	61.2
	878	6	39.97	7.34	12.26	−.66	58.91	11.63	701	10.42	89.6
	878	7	28.25	9.27	12.26	−.86	48.92	11.46	804	9.91	86.5
	879	8	8.36	12.43	12.26	−1.23	31.82	12.91	964	7.73	59.9
	990	1	67.12	.23	12.26	−.00	79.61	8.31	0	.00	.0
	995	2	76.61	1.13	12.26	−.04	89.96	13.22	164	3.72	28.1
	986	3	72.09	3.50	12.26	−.29	87.56	18.97	464	10.24	53.9
	984	4	64.41	5.37	12.26	−.46	81.58	20.30	588	12.09	59.6
986	988	5	56.05	7.57	12.26	−.67	75.21	20.05	707	13.42	66.8
	981	6	47.23	8.95	12.26	−.84	67.58	20.05	798	13.64	67.8
	985	7	39.55	10.40	12.26	−1.02	61.19	20.05	873	13.48	67.2
	982	8	30.06	11.98	12.26	−1.20	53.10	19.83	949	12.70	64.0
	985	9	19.21	13.79	12.26	−1.46	43.80	19.42	1,045	11.55	59.4
	982	10	9.04	15.14	12.26	−1.59	34.85	19.20	1,094	9.61	50.1
	1,086	1	82.26	.00	12.26	−.00	94.52	13.29	0	.00	.0
	1,083	2	92.89	.45	12.26	−.01	105.59	14.35	93	2.47	17.2
	1,075	3	93.11	1.13	12.26	−.06	106.44	17.95	211	5.66	31.5
	1,068	4	90.85	2.71	12.26	−.22	105.60	22.19	404	10.75	48.4
1,073	1,070	5	82.04	5.42	12.26	−.47	99.25	25.17	593	14.85	58.9
	1,074	6	74.35	7.57	12.26	−.69	93.49	26.58	716	16.89	63.5
	1,071	7	63.28	9.61	12.26	−.90	84.25	25.78	825	17.54	68.0
	1,069	8	50.62	11.64	12.26	−1.14	73.38	26.12	927	17.16	65.6
	1,069	9	32.32	14.46	12.26	−1.50	57.54	24.69	1,058	15.34	62.1
	1,067	10	11.75	18.08	12.26	−1.93	40.16	23.77	1,202	12.18	51.2
	1,162	1	112.44	.00	12.26	−.00	124.70	16.46	0	.00	.0
	1,165	2	112.77	.23	12.26	−.00	125.26	17.04	48	1.52	8.9
	1,149	3	111.42	1.13	12.26	−.05	124.76	20.98	199	6.27	29.9
	1,150	4	102.60	4.92	12.26	−.43	119.35	29.34	564	16.98	57.8
	1,154	5	94.69	6.55	12.26	−.60	112.90	30.89	671	19.12	61.7
1,155	1,148	6	90.85	7.57	12.26	−.70	109.98	31.22	727	20.18	64.5
	1,153	7	83.39	9.04	12.26	−.86	103.83	31.77	805	21.09	66.3
	1,153	8	70.06	11.75	12.26	−1.17	92.90	32.89	939	22.01	66.8
	1,155	9	44.97	15.93	12.26	−1.71	71.45	31.80	1,126	20.30	63.7
	1,155	10	33.00	18.36	12.26	−1.98	61.64	31.84	1,219	18.96	59.6
	1,161	11	21.70	19.55	12.26	−2.24	51.27	30.55	1,280	16.55	54.2

DISCUSSION OF TYPICAL CHARACTERISTIC CURVES

A typical characteristic diagram, including a capacity-head curve an efficiency curve, brake and water horsepower curve, and a speed curve, is shown as Figure 9, page 241, representing the various characteristics for pump No. 1 when run at an average speed of 898 revolutions per minute.

CAPACITY-HEAD CURVE

The capacity-head curve represents the relation between the total heads pumped against and the corresponding quantities of water in gallons per minute which the pump is capable of delivering when operated (in this instance) at an average speed of 898 revolutions per minute. The pump was started with zero discharge, i. e., the gate-valve control was closed and an initial total head of 128.6 feet was attained (see Table I). As the valve was slowly opened, the head gradually increased until a flow of 318 gallons per minute was reached with a total measured head of 134 feet. The head then dropped gradually, upon further opening of the control valve, until it came again to the measured total head of 129.3 feet, but with the discharge increased to 468 gallons per minute.

Thus it is seen that in this portion of the capacity-head curve there are two capacities of the pump for heads ranging from 128.6

to 134 feet for the average speed of 898 revolutions per minute. The stability of the operation of the pump between these limits is due to the balancing effect of the friction in the discharge pipe. As the head lowers, the capacity increases until a point is reached where the control-gate valve is wide open. At this point the discharge is maximum at 1,210 gallons per minute. The head would have been zero at this point of opening of the gate valve if the pump had been discharging into the air instead of raising the water high enough to pass through the measuring weir. Under such conditions, with zero head, no useful work would have been performed by the pump.

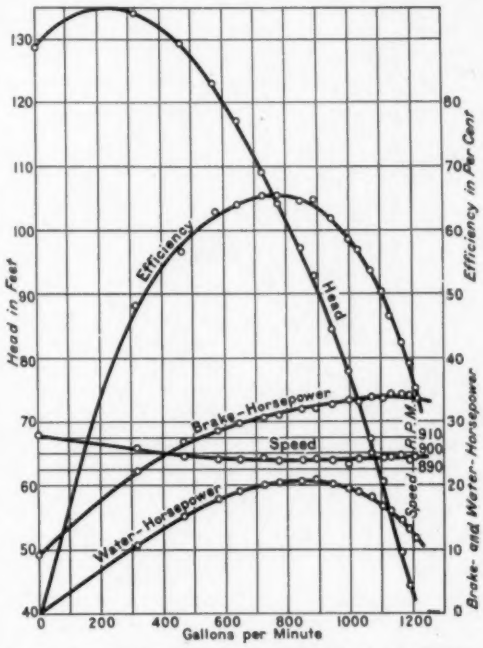


FIG. 9.—Characteristic curves, pump No. 1, 3-stage, 24-inch diameter, average speed 898 r. p. m.

EFFICIENCY CURVE

With no flow from the pump at the start, no useful work is being done by the pump, and hence the efficiency or ratio of output to input of power is zero. The efficiency curve therefore starts at zero. As the gate is opened, the capacity becomes greater and the efficiency increases very rapidly until a point of maximum efficiency of 65.4 per cent is reached. At this point the capacity is 775 gallons per minute. When the maximum capacity is reached the head is zero, with no useful work being done by the pump, and the efficiency also is zero.

A point of interest in connection with this curve is that for a given efficiency there are two heads with their respective capacities. For example: With an efficiency of 60 per cent, the corresponding heads are 127 feet and 78 feet, with capacities of 510 and 985 gallons per minute, respectively. The data show a wide range of heads and capacities for efficiencies of 60 per cent or greater. By referring to Figure 9, the range of heads and capacities may be noted for various efficiencies of 40 per cent and over.

BRAKE-HORSEPOWER CURVE

From this curve the size of prime mover to select can be determined, and the load which will be carried for any given set of con-

ditions ascertained. The brake-horsepower curve does not start at zero as do the capacity-head and efficiency curves, as indicated in Figure 9. For zero flow, the power consumed amounted to 9.48 horsepower, and the pump speed was 912 revolutions per minute, although the pump did no useful work. The curve reaches a maximum at 34.3 horsepower, with a total head of 56.3 feet and a flow of 1,130 gallons per minute. At the point of maximum capacity, the power output has decreased to approximately 33.7 horsepower and 39 feet head. This is a very important factor in connection

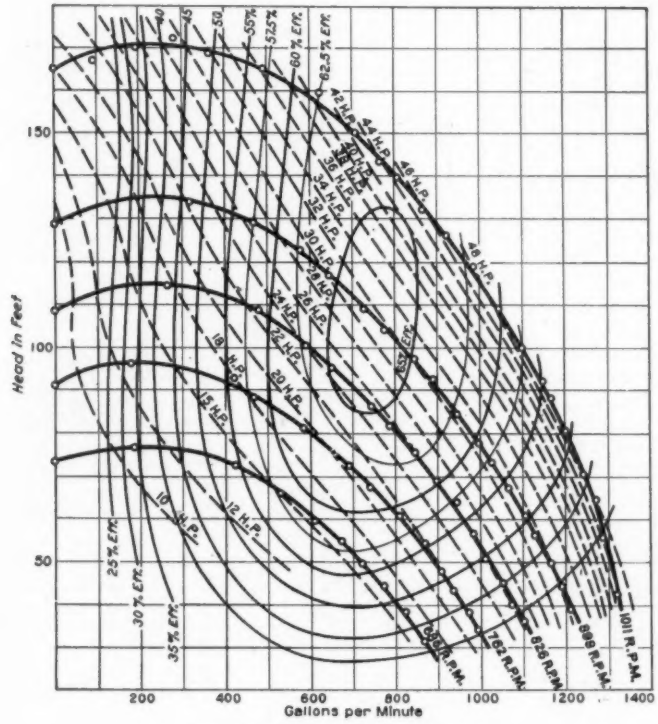


FIG. 10.—Performance curves, pump No. 1

with tests, as it shows that with a prime mover of 35 horsepower overload conditions would never exist at this speed, viz, 898 revolutions per minute. If the speed of the pump were increased as shown in Table I, new limits of capacity would exist. In any case, the pump should be operated so as to give an efficiency in excess of 50 per cent.

SPEED CURVE

The speed curve is the indicator of the proper speed at which to operate the pump to give best efficiency for a given head and capacity. This is a detail, already pointed out in this report, which must not

be neglected. Maximum speed occurs at the point of zero discharge, and in this particular instance the speed gradually lowers as the capacity of the pump increases.

ISO-EFFICIENCY CURVES

For practical use the data, first plotted in the form of characteristic curves, have been translated into the form of performance curves (figs. 10, 11, 12, 13, 14, 15, and 16).

In the selection of a pump, the pumping head is the first consideration; next is the desired capacity. For example: Assume that the pumping head is 105 feet and the desired capacity is 800 gallons per minute. With a 24-inch, 3-stage pump of the make corresponding

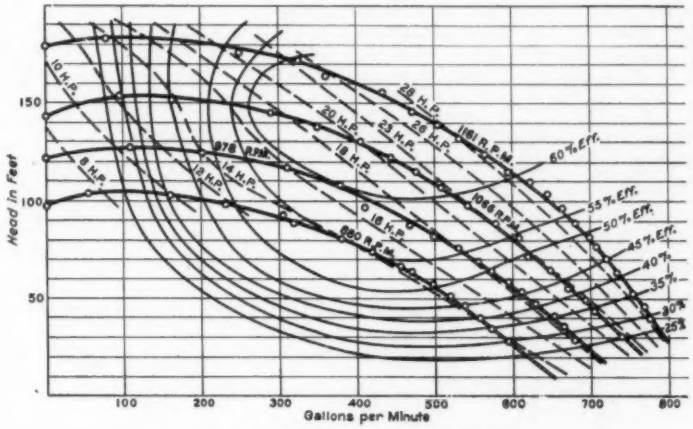


FIG. 11.—Performance curves, pump No. 2

to curves in Figures 9 and 10, an average speed of 898 revolutions per minute would give the best efficiency. At this speed a range of capacity of 500 to 1,000 gallons per minute would be possible without reducing the efficiency much below 60 per cent. For the set of conditions assumed, the power for 800 gallons per minute would be approximately 31.5 horsepower.

The curves emphasize the importance of considering the efficiency of the pump in relation to its speed. Intending purchasers should be impressed with the fact that the various types and makes of pumps have different characteristics, and that a hydraulic engineer, well versed in the performance of pumps, should be consulted before expensive pumping equipment is bought.

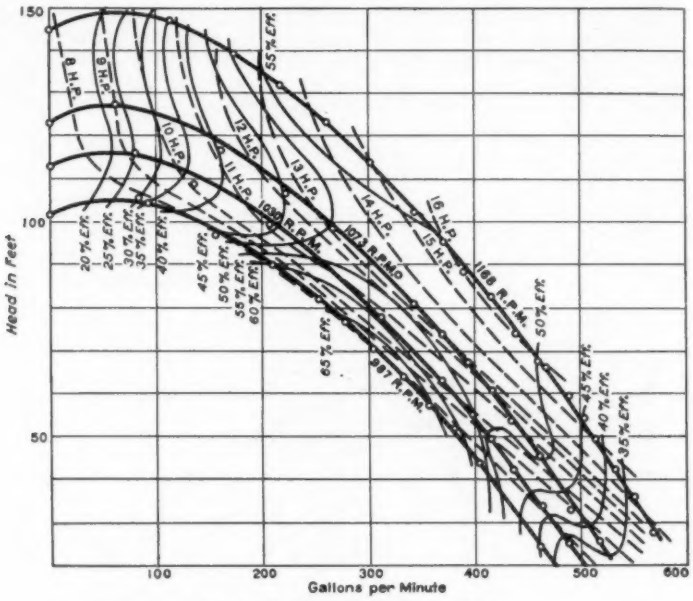


FIG. 12.—Performance curves, pump No. 3

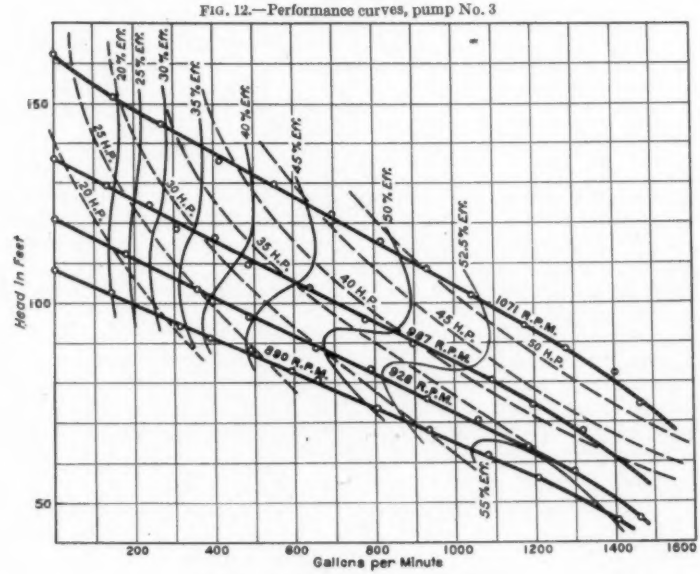


FIG. 13.—Performance curves, pump No. 4

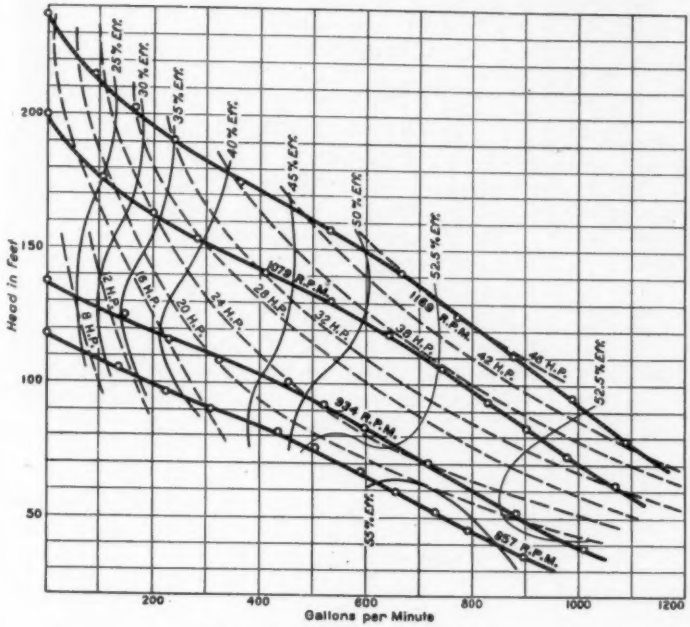


FIG. 14.—Performance curves, pump No. 5

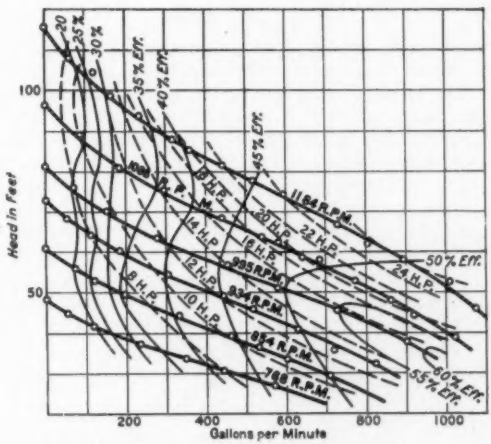


FIG. 15.—Performance curves, pump No. 6

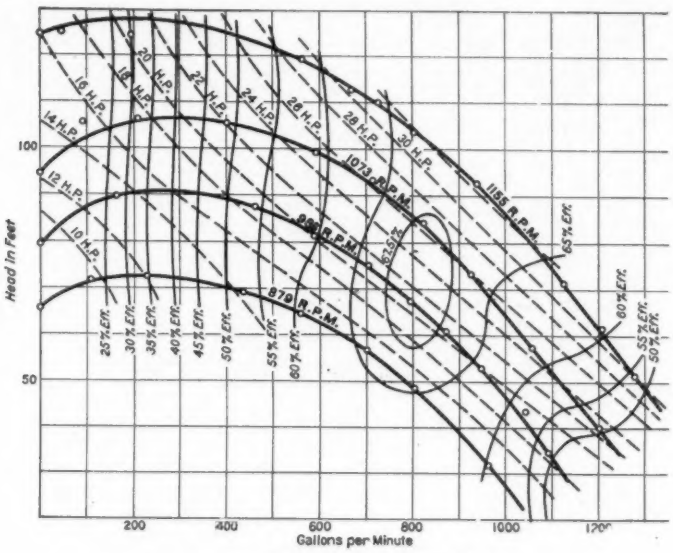


FIG. 16.—Performance curves pump No. 7

COLLOIDAL SILICA AND THE EFFICIENCY OF PHOSPHATES[1]

By P. L. GILE, *Chief, Division of Soil Chemistry*, and J. G. SMITH, *Assistant Chemist, Bureau of Soils, United States Department of Agriculture*

INTRODUCTION

General fertilizer practice recognizes in a vague way that there are reactions between soils and phosphatic fertilizers which affect the efficiency of the fertilizers. Bone meal, for instance, is recommended for use on open soils which are not too dry (*39, p. 169*),[2] and for light soils not too deficient in phosphoric acid (*32, p. 320*). Floats are recommended by Wheeler (*39, p. 173*) for acid soils high in organic matter, and by Schneidewind (*32, p. 321*) for acid high-moor soils but not for mineral soils. Acid phosphate, according to Schneidewind (*32, p. 297*), is adapted to all the "better" and heavier soils. However, the extent to which a phosphate is dependent on the character of a soil is probably not sufficiently appreciated.

EFFECTS OF SOILS ON THE EFFICIENCY OF PHOSPHATES

A complicated series of reactions follows the application of a phosphate to a soil; the nature and extent of the reactions must vary in different soils; and it is to be presumed that these reactions will affect the availability or efficiency of the fertilizer. Attempts have been made, by extracting the soil with various solvents, to determine what happens to a phosphate after it is incorporated in the soil (*13, 40*). But it has not been possible to follow the reactions in the laboratory far enough or precisely enough to determine just how the fertilizing efficiency of a phosphate will be affected. Evidence that the soil influences the efficiency of phosphates is therefore derived chiefly from experiments with plants.

That soils affect the efficiency of the various phosphates differently is indicated by many experiments in which the fertilizing values of the soluble and insoluble phosphates are compared. It has been shown frequently that rock phosphate or bone meal may be almost as effective as acid phosphate on one type of soil but quite ineffective as compared with acid phosphate for the same crop on another type of soil. Variations in the crop increase produced by a given quantity of rock phosphate on different soils are so great that it seems quite improbable that variations in the relative efficiency of the two classes of phosphates are due in all cases to differences in their so-called "secondary" effects on the soil; that is, in decreasing acidity, supplying lime, and the like. The low efficiency of rock phosphate in quartz sand and its high efficiency in some soils indicate that in certain soils reactions take place which enhance the "availability" of the phosphate—increase the supply of phosphorus acting as a plant nutrient.

[1] Received for publication September 29, 1924; issued September, 1925.
[2] Reference is made by number (italic) to "Literature cited," p. 259.

While the influence of soil conditions on the comparative efficiency of insoluble and soluble phosphates has been a subject of many investigations, the influence of the soil on the absolute efficiency of a soluble phosphate has received comparatively little attention. It has been known since the early days of soil science that the phosphorus of soluble phosphates is fixed by the soil and that some of the phosphorus is available after it is fixed. But we are comparatively ignorant of how much of the "fixed" phosphoric acid is available and how much remains unavailable in different soils. The following facts indicate that in many soils a considerable part of the soluble phosphorus is rendered at least temporarily unavailable: The small proportion of the applied phosphoric acid which is recovered in the crop; the loss in efficiency of phosphates remaining in unplanted soils; and the variation in the quantity of soluble phosphate required to produce a given crop increase on different soils.

When fertilizers containing soluble forms of nitrogen or potassium are applied, not in excess, to soils responding to these ingredients, 60 to 90 per cent of the quantities applied are commonly recovered in the crop (*36*, *37*, *23*). The recovery of phosphoric acid, however, frequently amounts to only 10 to 20 per cent (*26*, *14*). It might be held that the low recovery of phosphoric acid is due to only a part of the phosphorus being required as a plant nutrient, the remainder of the phosphorus being effective in increasing growth in other ways than in supplying available phosphorus to the plant. Some would hold, for instance, that soluble phosphates promote growth in some soils by reducing toxic, soluble aluminum in the soil (*3*, *2*). It seems possible, however, that soluble aluminum is injurious only as it renders phosphate unavailable to the plant. The fact that relatively high recoveries of soluble phosphates may be made by plants growing in quartz sand indicate that the low recoveries from many soils are due to interaction between the phosphate and certain soil constituents (*26*).

Pot experiments have shown that in some soils an appreciable loss in the efficiency of acid phosphate takes place when the phosphate is applied a few weeks in advance of planting, the loss occurring in the bare soil maintained at the optimum moisture content (*8*). It amounted in 30 days to approximately 40 per cent of the efficiency of acid phosphate applied immediately before planting. Since a crop does not abstract much phosphoric acid per acre until it has attained some size, it is evident that there may be an appreciable loss in the efficiency of acid phosphate applied under the usual conditions.

A further indication that certain soils render soluble phosphates partially unavailable is the fact that very different quantities of acid phosphate may be needed to produce the same increased yield on two different soils, even in cases where the two soils give approximately equal increases in yield with different maximum quantities of the phosphate. In some instances this may reasonably be attributed to different weather conditions, to different cultural methods, to the use of different nitrogenous fertilizers, and the like. Variability in the response of a crop attributable to such conditions, however, can be largely eliminated in pot experiments. And a marked influence of the character of the soil on the quantity of phosphate required to produce a given crop increase is observed in pot experiments also.

Thus there is considerable evidence that the reactions taking place when a phosphate is added to a soil may increase or decrease the efficiency of different phosphatic fertilizers, and that these reactions vary in different soils. Little is definitely known about these reactions, although Liebscher (*21, p. 208*) and subsequent investigators have pointed out that soils high in iron and aluminum usually respond to phosphate fertilization, and many have shown that calcium carbonate influences the efficiency of the phosphates (*17, 33, 34, 28, 8*). Consequently, although we have a general idea of some types of soils which respond poorly to certain phosphates, we can not predict with any certainty from a laboratory examination of a soil what its effect will be on any particular phosphate. A determination of the soil constituents and the soil conditions affecting the availability of the different phosphates is obviously an essential step toward scientific fertilizer practice.

It may well be that the properties of a soil affecting the efficiency of phosphates are largely localized in the colloidal material. The absorptive and base-exchange properties of the soil, which are almost exclusively a function of the colloidal material, might be expected to influence the solubility or decomposition of the relatively insoluble phosphates. Although Gedroiz (*4*) has suggested that insoluble phosphates are more efficient in adsorptively unsaturated soils, the influence of the soil colloidal material on the efficiency of phosphates has not been directly studied.

The soil colloidal material is a complex substance or mixture of substances. It is quite variable in composition but is made up chiefly of silicon, aluminum, iron, and organic matter. It also contains small amounts of magnesium, calcium, potassium, sodium, manganese, titanium, phosphorus, chlorine, and sulphur (*30*). The condition of the elements in the colloidal material has not been definitely determined, but the constituents may be regarded as forming a mixed gel similar in nature to the artificial gels of silica, iron, alumina, etc.

Some results obtained in this laboratory indicate that a mixed gel such as occurs in soils may have certain properties not possessed by simpler gels. Nevertheless, it seemed advisable to determine the influence of relatively simple gels on the efficiency of phosphates preliminary to dealing with the colloidal material of different soils. The effects of an iron gel alone and of an alumina gel mixed with silica gel have been tested by others. Prianishnikov (*27, p. 36*) found that hydrous ferric oxide depressed the efficiency of bone meal markedly in a sand culture experiment with barley. In an experiment of Pfeiffer and Blanck (*25*), the addition of a mixture of alumina and silica gels to a sand culture depressed the quantity of phosphoric acid assimilated by yellow lupines from potassium acid phosphate. The influence of a silica gel on the efficiency of rock phosphate and acid phosphate is shown in the following experiments.

INFLUENCE OF COLLOIDAL SILICA ON PHOSPHATE EFFICIENCY, AS SHOWN BY THE GROWTH OF MILLET

It was desirable to determine the effect of colloidal silica on the efficiency of rock phosphate and acid phosphate under the simplest conditions possible. Since organic matter, other colloids, and reactive soil minerals might complicate the effect, the test was conducted in a fairly pure quartz sand (99.5 per cent silica).

The colloidal silica used in these tests was a silica gel prepared chiefly by M. S. Anderson, of the Bureau of Soils, by treating a sodium silicate solution with H_2SO_4 and HCl until a strongly acid reaction persisted. The precipitate was then washed many times until the wash water had the same reaction as the distilled water used (P_H6.2). It was then dried on the steam bath and ground to pass a 1-millimeter sieve.

A mixed gel containing silica, alumina, iron, and small quantities of lime, magnesia, and potash, was also tested in one pot for comparison with the pure silica gel. This was prepared by adding the chlorides of aluminum, iron, etc., to a sodium silicate solution in such proportions that the resulting mixture contained silica and the various bases in the same proportions as the average soil colloidal matter. The resulting gel was washed until the wash waters were free from chlorides. The washing apparently removed a considerable part of the lime, magnesia, potash, and soda, and a small part of the iron and alumina.

Golden millet (*Chaetochloa italica*) was used as the test crop. This responds markedly to phosphoric acid fertilization, and, like the other Gramineae, it has a low "feeding power" for insoluble phosphates. Glazed earthenware pots of 1 gallon capacity were used as containers. In each pot was placed 5,030 grams of sand, which was maintained at a moisture content of 8 per cent of the dry weight by the addition of distilled water, the pots being weighed daily.

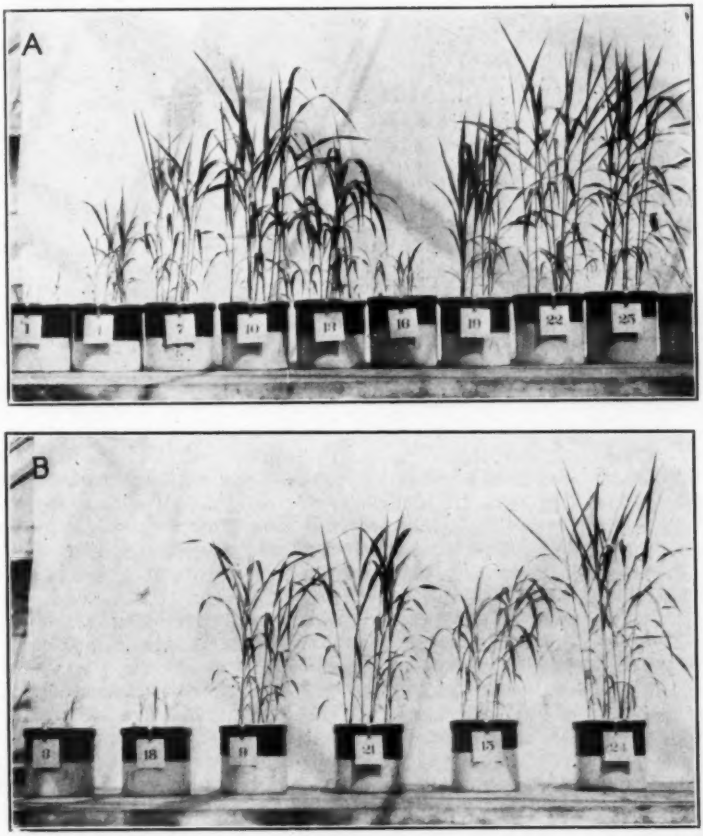
The special additions to the different pots—rock phosphate, acid phosphate, and silica gel—were thoroughly mixed with the upper half of the sand in the pot prior to planting. The rock phosphate used was a Florida pebble rock ground to pass a 100-mesh sieve. It contained 31.63 per cent of total phosphoric acid. The acid phosphate contained 18.72 per cent of available and 19.74 per cent of total phosphoric acid. Both phosphates were applied on the basis of the total phosphoric acid.

Each pot in the experiment received the following salts, applied in solution, as a basic fertilization to supply all essential nutrients except phosphorus: $Ca(NO_3)_2.4H_2O$, 1.070 grams; $(NH_4)_2SO_4$, 0.491 grams; KCl, 0.446 grams; $MgSO_4.7H_2O$, 0.914 grams; $Fe_2(C_4H_4O_6)_3H_2O$, 0.074 grams.

Seven millet plants were grown in each pot from July 29 to August 31, 1923. At the end of 33 days of growth the plants were in the joint stage, and those in the pots receiving the largest amounts of acid phosphate appeared about as large as could be grown without danger of having further growth restricted by size of pot or supply of basic nutrients. The pots were kept in a glass house and the order of the pots was shifted daily. The appearance of the plants well supplied with P_2O_5 was normal at all times (pl. 1).

The special treatments of the different pots and the oven-dry weight of the plants harvested are shown in Table I. Most of the special treatments were triplicated.

The greenhouse of the Division of Soil Fertility Investigations, Bureau of Plant Industry, was used for the conduct of this experiment.



A.—1, No phosphate; 4, acid phosphate, 0.03 gram P_2O_5; 7, acid phosphate, 0.06 gram P_2O_5; 10, acid phosphate, 0.12 gram P_2O_5; 13, rock phosphate, 0.24 gram P_2O_5; 16, no phosphate, silica gel, 50 grams; 19, acid phosphate, 0.06 gram P_2O_5, silica gel, 50 grams; 22, rock phosphate, 0.24 gram P_2O_5, silica gel, 50 grams; 25, rock phosphate, 0.24 gram P_2O_5, silica gel, 150 grams

B —3, No phosphate; 18, no phosphate, silica gel, 50 grams; 9, acid phosphate, 0.06 gram P_2O_5; 21, acid phosphate, 0.06 gram P_2O_5, silica gel, 50 grams; 15, rock phosphate, 0.24 gram P_2O_5; 24, rock phosphate, 0.24 gram P_2O_5, silica gel, 50 grams

TABLE I.—*Influence of colloidal silica on efficiency of acid phosphate and rock phosphate in sand, as shown by growth of millet*

Pot Nos.	Differential treatment of pots			Weight of plants above ground				Relative efficiency of the phosphoric acid (phosphoric acid of acid phosphate without silica gel=100)
	Kind of phosphate applied	Phosphoric acid (P_2O_5) applied per pot	Colloidal silica applied per pot	Oven-dry weight of plants per pot			Average oven-dry weight of plants per pot	
		Gram	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	
1, 2, 3	No phosphate		None	0.07	0.06	0.09	0.07	
4, 5, 6	Acid phosphate	0.03	do	1.35	1.09	1.27	1.24	100
7, 8, 9	do	.06	do	4.61	4.20	4.48	4.43	100
10, 11, 12	do	.12	do	9.17	9.56	8.53	9.09	100
13, 14, 15	Rock phosphate	.24	do	4.30	2.56	4.64	3.83	23
16, 17, 18	No phosphate		50 silica gel	.32	.34	.15	.27	
19, 20, 21	Acid phosphate	.06	do	4.40	5.55	5.12	5.36	119
22, 23, 24	Rock phosphate	.24	do	9.79	8.61	9.99	9.46	51
25	do	.24	150 silica gel	9.10			9.10	49
26	do	.24	45 "mixed" gel.	.11			.11	0

The increase in growth with increasing quantities of P_2O_5 from acid phosphate without silica gel shows the extent to which growth was dependent upon the supply of phosphoric acid. Since the increased growth was practically a straight-line function of the quantity of acid phosphate applied, it is possible, by interpolating on the curve, to calculate, with very slight error, the growth that would have been produced by any quantity of P_2O_5 from acid phosphate below 0.12 gm. The efficiency of the P_2O_5 of rock phosphate with and without silica gel and of acid phosphate with silica gel can therefore be calculated against that of acid phosphate alone on the basis of the quantities of phosphoric acid required to produce equal increases in growth as previously suggested (7).

It is apparent that 50 grams of silica gel per pot increased the efficiency of the P_2O_5 in acid phosphate slightly, raising it from 100 to 119, while the gel more than doubled the efficiency of the P_2O_5 in rock phosphate, increasing it from 23 to 51 as compared with 100 for the P_2O_5 of acid phosphate. The 150-gram application of silica gel gave practically the same increase in efficiency of rock phosphate as the 50-gram application, within experimental error. The mixed gel containing iron and alumina completely nullified the efficiency of rock phosphate. Where no phosphate was applied the silica gel increased growth by 0.20 gram. This increase is equivalent to what would have been produced by 0.0045 gram of P_2O_5 from acid phosphate; it may reasonably be ascribed to the gel rendering the phosphorus impurities in the quartz sand, in the nutrient salts, and in the containers used more available to the plants.

The results just given indicate that colloidal silica, in a simple medium of quartz sand and nutrient salts, increases the efficiency of acid phosphate slightly and that of rock phosphate greatly. It is believed that the beneficial action of the silica gel lies in increasing the quantity of P_2O_5 in solution or the rate at which it goes into solution. A quite different explanation of the action of colloidal silica in increasing the growth of certain crops in soil and sand

cultures was recently offered by Lemmermann and Wiessmann (*20*). They suggested that the beneficial effect of certain forms of silica is due, not to its action on the soil, but to a direct action on the plant. This view will be discussed later.

Analyses were made of the plants where sufficient material was available, by the methods of the Association of Official Agricultural Chemists (*1*). The results are given in Table II.

TABLE II.—*Assimilation of silica and phosphoric acid by millet grown with acid phosphate or rock phosphate with and without silica gel*

Pot Nos.	Differential treatment of pots	In the oven-dry substance of the plants			In the pure ash		Present in 7 plants (from 1 pot)		Of total P_2O_5 supplied, percentage present in plants above ground
		Pure ash, CO_2, and sand free	Silica (SiO_2)	Phosphoric acid (P_2O_5)	Silica (SiO_2)	Phosphoric acid (P_2O_5)	Silica (SiO_2)	Phosphoric acid (P_2O_5)	
		P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*Gm.*	*Gm.*	*P. ct.*
7, 8, 9	0.06 gram P_2O_5, acid phosphate	9.27	0.96	0.40	10.33	4.07	0.0426	0.0177	29.5
10, 11, 12	0.12 gram P_2O_5, acid phosphate	6.48	.59	.47	9.14	7.34	.0536	.0427	35.7
13, 14, 15	0.24 gram P_2O_5, rock phosphate	15.09	4.39	.59	29.05	4.02	.1681	.0226	9.4
19, 20, 21	0.06 gram P_2O_5, acid phosphate, 50 grams silica gel	9.66	2.54	.43	26.34	4.49	.1361	.0230	38.3
22, 23, 24	0.24 gram P_2O_5, rock phosphate, 50 grams silica gel	8.78	3.08	.54	35.10	6.02	.2914	.0511	21.3
25	0.24 gram P_2O_5, rock phosphate, 150 grams silica gel	8.40	2.74	.57	32.47	6.55	.2493	.0519	21.6

The percentages of phosphoric acid in the dry substance generally increased with the growth. However, the plants grown with rock phosphate contained a somewhat higher percentage of phosphoric acid in the dry substance than the plants grown with acid phosphate, although they contained approximately the same percentage of phosphoric acid in the ash as the acid-phosphate plants of equivalent growth. The percentage of phosphoric acid in the plant is doubtless influenced to some extent by the rates at which the plants grew during different stages of their development, or by slightly different stages of maturity at the time of harvesting.

During the latter part of the growing period the rock-phosphate plants appeared to be picking up slightly on the acid-phosphate plants; owing to increased excretion of carbon dioxide or to some other cause, they doubtless obtained phosphorus at a somewhat faster rate toward the end of the growing period than at the start. The assimilability of the phosphorus of acid phosphate, on the other hand, might have been more constant. Such differences would account for the different percentages of P_2O_5 in the dry substance of the plant.

Addition of silica gel increased the percentage of silica in the acid-phosphate plants by more than one and a half times. In the case of the rock-phosphate plants, however, the silica gel diminished the percentage of silica in the dry substance and raised the silica in the ash relatively little.

The quantities of phosphoric acid and silica present in seven plants (the yield from one pot) are significant in indicating how silica gel increased the yield. It will be noted that the quantities

of silica removed by the crop bear little or no relation to the yields of the differently treated pots given in Table I. The quantities of phosphoric acid removed, however, approximate fairly closely the relative yields produced by the various treatments. It would thus appear that the silica in the plant was not a determining factor in the yield and that the phosphoric acid assimilated probably was the limiting condition of growth.

The last column in Table II shows that the application of silica gel promoted the assimilation of phosphoric acid from rock phosphate to a marked extent, while it increased the assimilation of phosphoric acid from acid phosphate comparatively little.

In a complex medium such as a soil, silica gel might promote the availability of rock phosphate without the increased availability being apparent through any test except that of the growing crop. However, in the simple medium of this experiment—quartz sand and soluble salts—an increased availability of rock phosphate should be revealed by an ordinary chemical analysis, since the quartz sand, except for slight impurities of iron and possibly other substances, would be without action on phosphoric acid, brought into solution. Laboratory tests were conducted on the solubility of rock phosphate simply with the nutrient salts used in the basic fertilization.

EFFECT OF COLLOIDAL SILICA ON THE DECOMPOSITION OF ROCK PHOSPHATE

The concentration and relative proportions of the nutrient salts added to the pots (basic fertilization) in the experiment just described of course underwent marked alteration with the growth of the plants. It was impossible, therefore, to determine the solubility or decomposition of rock phosphate in a solution representative of that present in the pots during all stages of the growth of the crop. However, the effect of silica gel in promoting the growth of plants supplied with rock phosphate was apparent when the plants were only a few inches high, and at this time the composition of the nutrient salts must have been essentially the same as at the start of the experiment. It was not expected that the quantities of phosphoric acid found in solution on shaking up rock phosphate with the nutrient salts and silica gel would correspond quantitatively to the quantities present in the pots at all times, but it was thought that the results obtained would indicate qualitatively the effect of silica gel on the solution or decomposition of rock phosphate in the pots.

In the first test, 0.5 gram of rock phosphate or 0.3 gram of acid phosphate was shaken up with a mixed salt solution or with a salt solution plus silica gel. The salt solution contained the equivalent of 1.25 grams of anhydrous salts in 250 cubic centimeters of water, the same salts being present in the same proportions as in the mixture used for the basic fertilization of the pot experiment. The silica gel added was the same as that used in the pot experiment. The mixture was shaken at intervals during seven days standing. It was then filtered through a Pasteur-Chamberland filter and P_2O_5 was determined in an aliquot of the filtrate, which was perfectly clear. Prior to precipitating the phosphorus, special precautions were taken to rid the filtrate of silica which might contaminate the phosphorus precipitate. Three dehydrations with HCl were made with intervening filtrations. The P_2O_5 was determined as $Mg_2P_2O_7$. The

quantities of P_2O_5 and SiO_2 found in solution,[3] calculated for the 250 cubic centimeters of solution, are shown in Table III, together with the special treatments of each flask.

TABLE III.—*Soluble phosphoric acid obtained from rock phosphate and acid phosphate in a mixed salt solution with or without silica gel*

Contents of flasks (in addition to 1.25 grams mixed salts and 250 cubic centimeters water)	Phosphoric acid (P_2O_5) in solution in 250 cubic centimeters	Silica (SiO_2) in solution in 250 cubic centimeters
	Gram	*Gram*
0.5 gram rock phosphate	0.0010	0.0021
0.5 gram rock phosphate, 5 grams silica gel	.0012	.0184
0.5 gram rock phosphate, 15 grams silica gel	.0019	.0207
0.5 gram rock phosphate, 30 grams silica gel	.0045	.0229
0.3 gram acid phosphate	.0344	.0025
0.3 gram acid phosphate, 15 grams silica gel	.0346	.0231

The 30-gram addition of silica gel appears to have markedly increased the quantity of phosphoric acid brought into solution from rock phosphate.[4] The increase produced by 15 grams of gel also appears real, but that attributable to the 5 grams of gel is probably less than experimental error. The solubility of the acid phosphate P_2O_5 in the salt solution was apparently not affected by the silica gel.

In order to check the accuracy of these results, a second test, similar to the first, was conducted with the rock phosphate. The method and materials of the second test were the same as those of the first, but the quantities of phosphate, gel, and salts were somewhat different; also most of the different treatments were duplicated. One flask (the last in the table) was included in which the mixed salts were varied solely by omitting the iron. The mixtures were allowed to stand 12 days with an occasional shaking. The results of this second test are given in Table IV.

TABLE IV.—*Soluble phosphoric acid obtained from rock phosphate in a mixed salt solution with or without silica gel*

Contents of flasks (in addition to 1.64 grams of mixed salts and 251 cubic centimeters water)	Phosphoric acid: (P_2O_5) in solution in 251 cubic centimeters		Silica (SiO_2) in solution in 251 cubic centimeters	
	Individual flasks	Average of duplicates	Individual flasks	Average of duplicates
	Gram	*Gram*	*Gram*	*Gram*
No phosphate, 31.3 grams silica gel	0.0011	0.0009	0.0228	0.0208
	.0006		.0187	
Rock phosphate containing 0.15 gram P_2O_5	.0006	.0006	.0023	.0022
	.0005		.0021	
Rock phosphate containing 0.15 gram P_2O_5, 31.3 grams silica gel	.0054	.0054	.0237	.0232
	.0054		.0227	
Rock phosphate containing 0.15 grams P_2O_5, 31.3 grams silica gel, no Fe in mixed salts	.0067	----------	.0215	----------

[3] The quantity of silica shown as "in solution" represents the quantity passing through the Pasteur-Chamberland filter. Part or all of this may have been present as a very finely dispersed sol rather than as a true solution.

[4] Similar results were obtained by Mattson (*24*, p. 77) on digesting a precipitated tricalcium phosphate with water suspensions of sphagnum peat, ceramic clay and quartz flour. The first two substances were particularly effective in decomposing the phosphate.

This test confirms the preceding one in showing that when rock phosphate is exposed to a mixed salt solution the addition of a fairly large quantity of silica gel markedly increases the quantity of phosphoric acid in solution. The omission of the small quantity of iron from the mixed salts apparently increases the quantity of phosphoric acid in solution. Doubtless the iron precipitates some of the phosphoric acid brought into solution by the action of the colloidal silica.

DISCUSSION OF RESULTS

The yields of plants in the pot experiment, where the phosphorus supply was the limiting factor, the quantities of phosphoric acid in the plants, and the laboratory determinations of the solubility or decomposability of the phosphates, all indicate that silica gel under the simple conditions of these experiments markedly increased the assimilability of the phosphoric acid in rock phosphate and influenced relatively little the assimilability of the phosphoric acid in acid phosphate. If the plant experiment had been conducted in a different medium or with a different basic fertilization, it is quite possible that the silica gel would have had a more or less marked effect on these two phosphates.

Lemmermann and Wiessmann (*20*) conducted many pot experiments in which a silica gel and other finely divided forms of silica were added to quartz sand and to soils. The phosphorus supply was obviously the chief factor limiting growth in these experiments and in all cases the silica gel increased the yield appreciably. As a result of their experiments Lemmermann and Wiessmann concluded that silica acts to a certain degree as a substitute for phosphoric acid (it is "phosphorsäuresparend") and that the action is within the plant, not on the soil. While the results of these experiments are not questioned, it is believed that the conclusion drawn is hardly justified, especially in view of the fact that the plants were not analyzed for either silica or phosphoric acid. The results obtained by these investigators could be explained either by assuming that silica acted as a substitute for phosphoric acid in the plant or that silica gel rendered the phosphorus in the medium more available.

The fact that plants which normally contain a high percentage of silica have, in certain experiments, been grown practically free from silica shows that silicon is not an essential nutrient such as nitrogen, phosphorus, or potassium. It is evident that almost all the silica assimilated by plants can be omitted without abnormally affecting growth.[5] Oats, the crop grown by Lemmermann and Wiessmann in most of their experiments, usually contain about 50 per cent of silica

[5] Sachs (*31, p. 288*) and Knop (*18, p. 501*) grew maize, and Kreuzhage and Wolff (*19*) grew oats, in nutrient solutions lacking silica, the plants containing only a fraction of the normal content of silica. Jodin (*16*) grew maize for four generations in this way. Rice, which normally contains about 10 per cent of silica in the dry matter (*5*) contained only 0.2 per cent of silica grown in a silica-free nutrient solution (*6, p. 508*). However, owing to experimental difficulties, it has not been shown whether good plants can be grown absolutely devoid of silica.

Several investigators have obtained increased growth by adding silica in some form to nutrient solutions or to the soil (*19, 29, 11, 12*). None of these experiments established that the increased growth was due to an increased assimilation of silica. The nutrient solution with and without silica used by Rautenberg and Kühn were of different compositions. Apparently none of the plants in Grégoire's experiments made a vigorous growth, and an increase was also obtained with alumina. The data considered by Hall and Morison were obtained from a field plot that had been treated with sodium silicate, an alkaline substance. The work of Livingston (*22*) and Jennings (*15*) indicate that growth may be altered in nutrient solutions by nonnutrient substances. While it has not been proved that plant growth is promoted by assimilation of silica, it is also yet to be proved that assimilation of silica does not promote growth.

in the ash of the whole plant (*38*). If growth is not abnormally diminished by the omission of most of this silica, it is hard to conceive of growth being promoted by a relatively small increase in silica over the average content.

It should be noted that Lemmermann and Wiessmann obtained significant increases only with pure silica gel, which is adsorptively active and contains no ions for base exchange. Kieselguhr, kaolin, various finely powdered silicas, and permutite, which contains exchangeable calcium, gave no increase. In view of the hypothesis they proposed, it would have been highly advisable to have determined which forms of silica increased the silica contents of the plants.

Experimental results reported in this paper confirm certain results of Lemmermann and Wiessmann, but contradict their hypothesis of the beneficial action of silica gel. Data given in Table I and II indicate that an increased assimilation of silica by the plant does not enable the plant to get along with less phosphoric acid.

Addition of silica gel to pots receiving 0.06 gram P_2O_5 from acid phosphate increased the growth of plants by 21 per cent, the quantity of P_2O_5 taken up by 30 per cent, and the quantity of silica assimilated by 219 per cent. If we attribute the slight increase in growth to the slight increase in the quantity of phosphoric acid assimilated the 219 per cent increase in silica taken up had no effect on growth. Plants grown with 0.24 gram P_2O_5 from rock phosphate made 14 per cent less growth than plants grown with 0.06 gram P_2O_5 from acid phosphate, although they took up 28 per cent more phosphoric acid and 295 per cent more silica. Here the increased assimilation of silica would seem to have diminished rather than enhanced the effectiveness of the phosphoric acid in the plant. Plants grown with 0.24 gram P_2O_5 from rock phosphate plus silica gel made 4 per cent more growth than plants grown with 0.12 gram P_2O_5 from acid phosphate and assimilated 20 per cent more phosphoric acid and 444 per cent more silica. In this case the slight increase in growth is more than accounted for by the phosphoric acid assimilated and no beneficial action of the 444 per cent excess assimilation of silica is apparent.[6]

While it seems very apparent that the addition of silica gel to the quartz sand and salts of our pot experiment increased the assimilability of the phosphoric acid of rock phosphate, a complete explanation of the action of the gel can hardly be offered on the basis of the work done. The laboratory tests with solutions indicate that the silica gel increased the assimilability of rock phosphate by increasing the quantity of phosphoric acid in solution. Possibly the gel increased the decomposition of the rock phosphate by adsorbing calcium hydroxide—one of the end products of the decomposition. It is also possible that the gel acted by adsorbing OH ions.[7] There was some evidence of this in tests made of the hydrogen-ion concentrations of the solutions in the different flasks. However, the water extracts of the various pots after the plants had been grown in them did not bear this out.

[6] It might be held that failure of the enhanced assimilation of silica to increase growth in this case was because growth was no longer limited by the phosphorus supply. However, the steep form of the curve obtained on plotting growth increase against 0.03, 0.06, and 0.12 gram P_2O_5 from acid phosphate would indicate that this was not the case.

[7] The experiments of Gordon and Starkey (*10, 35*) indicate that silica gel does not absorb an appreciable amount of Ca from neutral salts, especially from an acid solution, although alumina and iron gels may. According to Glixelli (*9*), silica gel absorbs OH ions and the hydroxides of Na, K, Ca, and Ba.

The action of silica gel in the simple medium of these experiments probably does not correspond closely to the action of the colloidal material which is present in a soil. The results do show that a substance of the same general physical, but not chemical, nature as the soil colloidal material may markedly increase the efficiency of a phosphate fertilizer. Previous studies have shown that other substances of the same general physical nature as the soil colloidal material (an iron gel and an alumina gel acting with a silica gel) may appreciably depress the efficiency of phosphate fertilizers. As previously mentioned, the soil colloidal material is a mixed gel containing many different elements but consisting chiefly of silica, iron, and alumina. It might be supposed that such a mixed gel would have an action on phosphates approaching that of a silica gel. Apparently this is not necessarily so. The mixed gel used in these experiments depressed the efficiency of rock phosphate to zero, which is all that would be expected of a pure iron gel. At first thought this seems rather surprising. It should be borne in mind, however, that while a pure iron or alumina gel has somewhat of an adsorptive power for electrolytes, it does not give an appreciable concentration of iron or alumina in solution when treated with a neutral salt. On the other hand, it was found by Anderson, of this laboratory, that the mixed gel which he had prepared gave a heavy concentration of iron and alumina in solution when treated with a fairly concentrated potassium chloride solution. The depressing action of the mixed gel in the writers' experiments was therefore probably due to the maintenance of sufficient iron and alumina in solution to preclude the presence of phosphate ions.

SUMMARY

In an experiment with millet grown in sand culture, the addition of silica gel greatly increased the growth of plants supplied with rock phosphate and only slightly increased the growth of plants supplied with acid phosphate. The addition of a mixed gel containing iron, alumina, and silica to a pot receiving rock-phosphate was marked by reduced growth as compared with the growth made in pots receiving no phosphoric acid.

Growth made by the plants in the different pots was approximately proportional to the quantities of phosphoric acid in the plants, but seemed to bear no relation to the quantities of silica taken up.

The beneficial action of silica gel on the growth of plants supplied with rock phosphate is ascribed to the increasing of the availability of this phosphate through increasing the quantity of phosphoric acid in solution. Similarly, the deleterious effect of the mixed gel is ascribed to a diminishing of the quantity of phosphoric acid in solution, owing to soluble iron and aluminum produced by base exchange.

This conclusion is substantiated by the quantities of phosphoric acid found in solution on shaking up rock phosphate with silica gel and a nutrient solution such as was used in the pot experiment.

Criticism is made of the idea that the increased growth of plants observed in similar experiments is due to an increased assimilation of silica which enables the plant to get along with less phosphoric acid.

The relation of the action of silica gel on the efficiency of phosphates to that of the soil colloidal material is discussed.

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BEST TIME FOR SOWING SILVER FIR IN THE NURSERY [1]

By J. V. HOFMANN

Forest Examiner, Wind River Forest Experiment Station

INTRODUCTION

Early attempts to grow silver fir (*Abies grandis*) at the Wind River Nursery, Carson, Wash., resulted in almost complete failure. One exception, however, was a successful sowing in the late spring. Since the previous losses were nearly all due to damping off, this singular success was attributed to the later date of sowing and the inactivity of the damping-off fungi after the soil had warmed. In order to correlate successful germination with time of sowing and soil temperature, the following experiment was undertaken.

METHOD

The unit seed bed area used was one-half of a standard 4 by 12 foot bed, making the unit 4 by 6 feet. The first bed was sown on November 16; the second on March 25 of the following spring, the earliest date it was possible to work the soil. The series was then continued at about 10-day intervals.

The seed used was gathered in the fall just before the first sowing, at Red Mountain, on the Columbia National Forest, at about 4,000 feet elevation. It was extracted by air drying where gathered, and was then shipped to the Wind River Nursery and stored in sacks in a cool, dry room. A cutting test on November 15 showed the seed to be 39 per cent good, 10 per cent wormy, and 51 per cent bad. Another cutting test March 21 following showed 36 per cent good, 12 per cent wormy, and 52 per cent bad. There were 12,550 seeds per pound. Sowing was at the rate of 2,500 good seeds per bed, based on weight and cutting test.

The fall-sown bed remained unshaded, as did the spring-sown beds, until the middle of June, when 50 per cent shade was applied. The shades were left off to prevent damping off and to produce a warmer soil in the seed beds.

The seed beds were not allowed to become dry but the watering was done at a time when the surface would dry soon after, to prevent damping off.

Soil-temperature readings were taken at 1-inch and 6-inch depths at intervals of about three days.

Germination, mortality, and survival counts were taken daily from April 17, one week before germination began, until August 15, the close of germination. These counts were made in three areas in each bed, each area being 2 feet by 6 inches. Hence 3 square feet were counted. These areas were permanently marked with pegs and cord at the time of sowing. The seedlings were marked with colored toothpicks, each color indicating a one-week age class. Causes of mortality were determined by cultures from dead or dying seedlings.

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Notes on terminal-bud formation and on second growth were taken September 3 and November 6.

After the close of the growing season weights and measurements of the top and root development of the seedlings, taken from 25 plants arbitrarily selected from each bed, were recorded.

RESULTS

A general correlation of soil temperature and germination of seeds is established. When the soil temperatures given in Figure 1 and the period of best germination in Table I are related, definite relations between soil temperature and germination are seen, although specific instances differ. The periods of best germination for the sowings of March 25 and April 4 are shown to be later than those for the sowings of April 13 and April 25. This condition emphasizes the danger of early spring sowing because of the long dormant period of the seed and the consequent exposure to damping off. The month of May was unfavorable to germination, and the periods of best germination were after the soil had warmed. Likewise the best germination period of the fall sowing came when the soil was warmest in the latter part of April and early May. The general indications of the spring sowings are that germination does not proceed at its best until the soil temperature is 70° F. or warmer to a depth of 6 inches. Surface temperature may be higher, but a cooler soil below the surface causes rapid and extreme fluctuations at the surface and the seed does not get the benefit of a high enough temperature for good germination.

The fall sowing resulted in the greatest germination and also the highest percentage of survival, as shown by Table I and Figures 2 and 3.

TABLE I.—*Effect of time of sowing on germination and bud maturity*

Time of sowing	Number of days before germination	2-week periods of best germination	Total germination per square foot	Percentage of buds mature	
				Sept. 3	Nov. 6
			Number	*Per cent*	*Per cent*
Nov. 16, 1917	At least 30 days in spring.	Apr. 23–May 6	52.5	88	100
Mar. 25, 1918	73	June 20–July 3	20.8	73	100
Apr. 4, 1918	64	June 21–July 4	28.2	85	100
Apr. 13, 1918	58	June 17–June 30	24.9	71	100
Apr. 25, 1918	53	do	23.8	23	100
May 4, 1918	51	June 24–July 7	29.1	24	100
May 20, 1918	35	do	28.2	6	98
May 31, 1918	38	July 15–July 28	27.2	11	95

The losses were due almost entirely to damping off, practically all those indicated by the cultures being caused by *Pythium debaryanum*. *Rhizoctonia* sp. was found in a few cultures later in the season.

Figure 2 shows that germination proceeded quite uniformly in each bed after it began, with the exception of the fall sowing. Although weekly records vary, all of the spring sowings were quite uniform in final germination, with slightly wider differences in survival, as shown in Figure 3. The dormant period of the seed in the seed bed was very much shorter in the later sowings. This is an important factor, as it reduces the period of exposure to damping off. The fall-

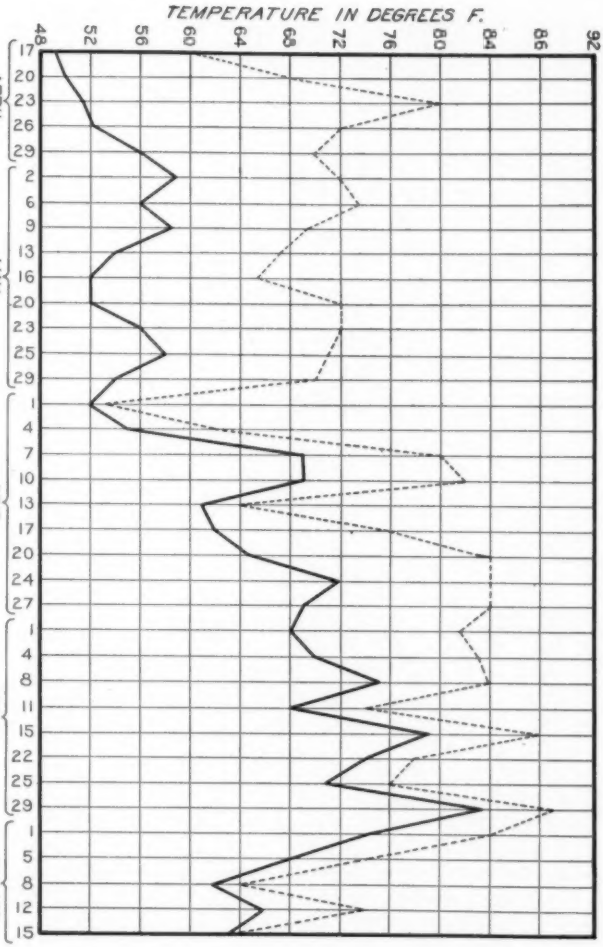

FIG. 1.—March of soil temperature at depths of 1 and 6 inches in the nursery, 1918

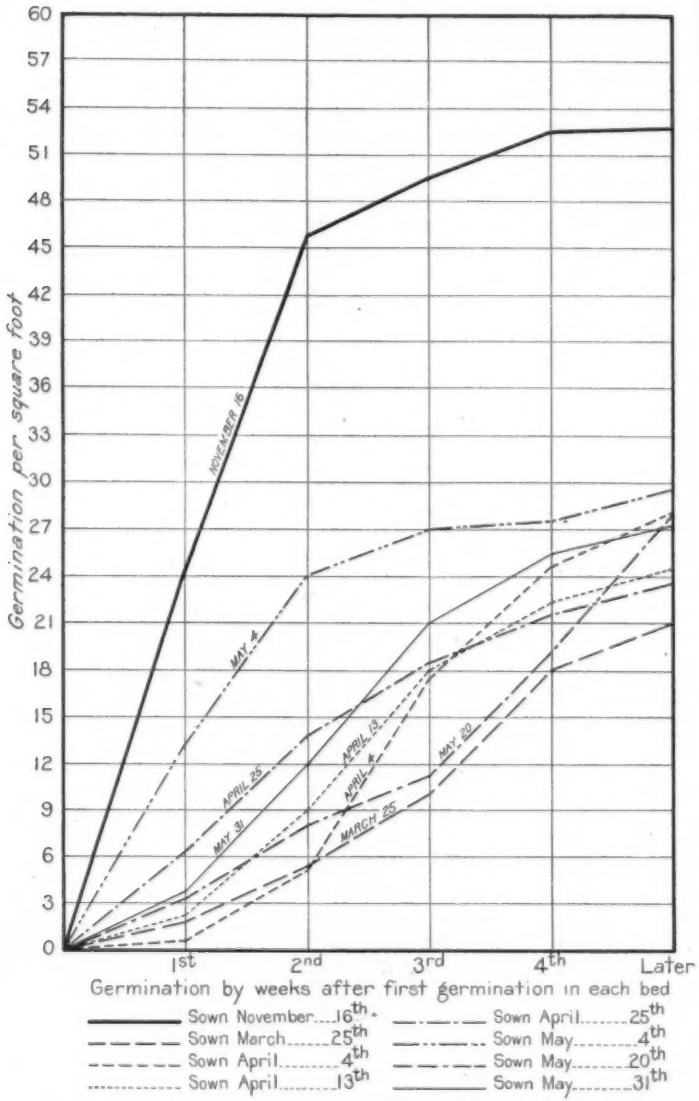

FIG. 2.—Germination of silver fir seed by weeks after first germination in each bed

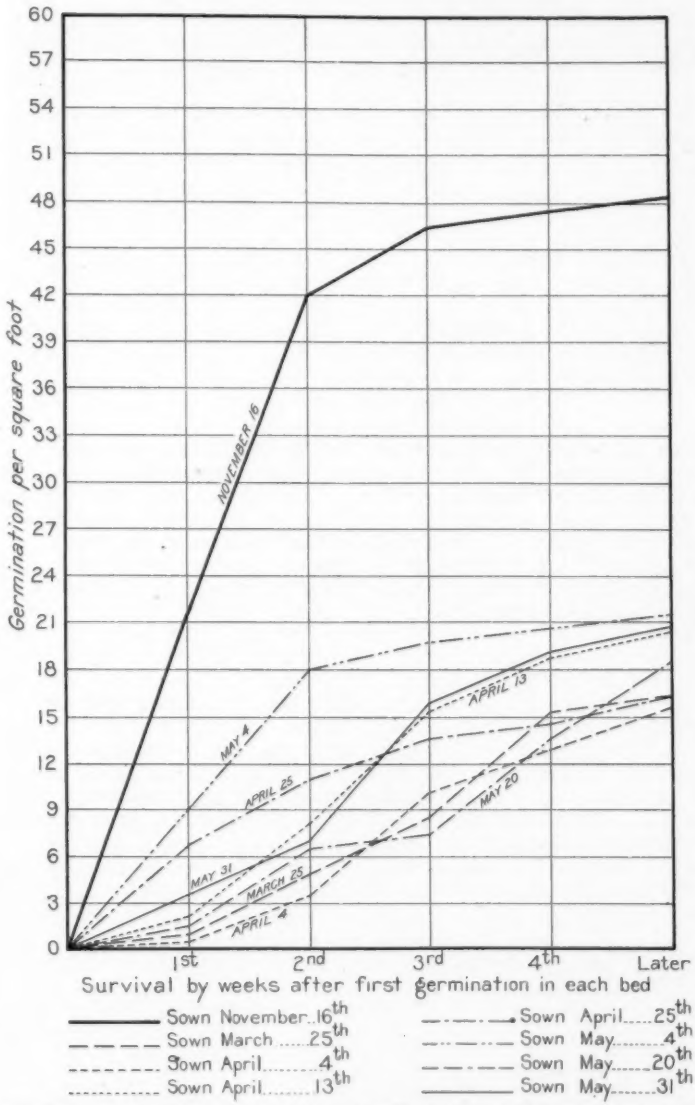

FIG. 3.—Survival of silver fir seedlings by periods in weeks following the first germination, fall and spring sowings.

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sown seedlings matured their buds earlier, although this season the buds of all of the seedlings matured. The first killing frost came in November, while usually the first frosts occur in early October. In an average fall the buds of most of the spring-sown seedlings would have been immature and subject to injury by frost. There was no tendency to develop second growth in any of the stock.

TABLE II.—*Effect of time of sowing on development of seedlings*

Time of sowing	Weight of entire root system	Weight of root system pruned to 5-inch length	Secondary roots	Length of root	Weight of top	Length of top
	Ounces	*Ounces*	*Number*	*Inches*	*Ounces*	*Inches*
Nov. 16, 1917	0.56	0.52	11	6.3	0.60	1.4
Mar. 25, 1918	.28	.28	2	3.8	.48	1.0
Apr. 4, 1918	.36	.36	4	5.6	.44	1.0
Apr. 13, 1918	.28	.28	2	4.1	.48	1.1
Apr. 25, 1918	.24	.24	2	4.8	.48	1.3
May 4, 1918	.24	.24	1	3.9	.40	1.0
May 20, 1918	.20	.20	1	4.2	.36	1.0
May 31, 1918	.16	.16	1	2.3	.28	1.0

The fall sowing produced by far the best developed plants, as shown in Table II. The plants were larger, more sturdy, and had a well-developed, branching root system. The plants of the spring sowings showed only normal variations, except the late sowings of May 20 and May 31. The uniform development of all of the other sowings was no doubt due to the delayed germination of the earlier sowings and the optimum germination of all of these sowings at approximately the same time.

SUMMARY

The results of this experiment show that the best developed and hardiest plants result from fall sowing. Simply on the basis of plant development fall sowing would be recommended, but the danger of damping off complicates the question. There is always the possibility of loss by damping off. Spring sowing should be done not earlier than the last week of April and not later than the second week in May, in an average season.

GIRDLING AS A MEANS OF REMOVING UNDESIRABLE TREE SPECIES IN THE WESTERN WHITE PINE TYPE[1]

By DONALD R. BREWSTER and JULIUS A. LARSEN, *Forest Examiners, Northern Rocky Mountain Forest Experiment Station, Forest Service, United States Department of Agriculture*

INTRODUCTION

Western hemlock (*Tsuga heterophylla*) and white or grand fir (*Abies grandis*), two shade-tolerating species which are found in more or less abundance in the western white pine type in northern Idaho and northwestern Montana, must usually be classified as undesirable species silviculturally, and for several reasons. In both species the liability to infection by heart-rot at an early age causes such a high percentage of defect that by the time the trees reach merchantable or saw-log size they are apt to be largely or entirely worthless. Even when sound, the material produced by these species is of poor quality and low value; and in stands in which they occur to any extent the stumpage yield per acre is materially decreased by the fact that they occupy space which might support more valuable species.

A particularly undesirable feature is the dense shade cast by these trees. The advance growth of hemlock and white fir seedlings and saplings which comes in under the mature trees has a tendency to monopolize the openings between the larger trees and to repel less shade-tolerant species. As a result, on cutting areas where seedlings, saplings, and larger trees of these species are present, it is difficult to obtain establishment and growth of reproduction of the more intolerant desirable species of western white pine (*Pinus monticola*) and western larch (*Larix occidentalis*).

In order to get maximum production of the most valuable species in future stands, the hemlock and white fir trees left standing on cut-over areas should be disposed of, and measures to prevent or hinder the reseeding of the ground by these species should be taken.

Possible methods of disposing of undesirable tree species include girdling, burning, and poisoning.

Killing trees by fire is particularly effective with thin-barked species, such as hemlock and white fir. Fire may be applied by burning piles of forest débris at the base of undesirable trees, or in thickets; or it may be applied as broadcast burning of slashings, which destroys most or all of the undesirable reproduction and larger trees. Broadcast burning, however, in most cases destroys also the valuable seed trees and trees left for increased growth, as well as most or all of the seed stored in the surface duff.

Poisoning, or introducing some toxic material into the tree through the sap, has been used to some extent for killing hardwood stump sprouts in farm clearings in the Eastern States. Although the poisoning method may very often be too expensive to be practicable, it offers possibilities worthy of investigation.

[1] Received for publication Oct. 28, 1924; issued September, 1925.

Girdling, an old and common method, consists of so severing the cambium and phloem of the tree that the nutritive communication between root and crown is permanently interrupted.

EARLY ATTEMPTS AT KILLING BY GIRDLING

As far as known to the writers, the first attempt, apart from general broadcast burning, for the specific purpose of destroying undesirable white fir and hemlock on Forest Service sales, was in 1910, on the Kaniksu National Forest. Here the hemlock and white fir were girdled in three seed plots, a belt of bark 2 to 3 feet high around the base of the tree being stripped off. The work was not done carefully and many strips of inner bark were left intact, connecting the root and the top. Consequently very few of the trees were killed and the wounded portion is gradually healing by the growth of new bark.

The objects of the girdling—to prevent seed production on the adjoining clean-cut area, and to lessen the shade in the seed plot—were both defeated, as the girdled trees produced heavy seed crops three years later, and the shade in the plots was not materially decreased.

The next work of the kind was on several cuttings in the white-pine type on the Coeur d'Alene National Forest, which was followed the next year by additional girdling on later sales. The method has since been specified for use in all white pine sales on the Coeur d'Alene Forest.

Two different forms of girdling were employed in these early trials. One was to strip off the bark around the base of the tree for a width of 2 to 3 feet, a method similar to that used on the Kaniksu National Forest. The other was to cut a notch 1 to 2 inches deep completely around the tree, with or without some stripping of the bark above or below the notch.

The stripping method was tried in the winter when the bark adhered so tightly that it would not peel but had to be cut off. The leaving of narrow strips of inner bark connecting the upper and lower ends of the wounds was, in many cases, unavoidable. The following year it was noticed that many of the hemlock and white fir trees which had been girdled by the notch or stripping methods still had green healthy tops and were bearing unusually heavy crops of cones. In order to determine whether the girdling had affected the fertility of these cones, two large hemlocks, one girdled by stripping and the other by notching, were cut, and a bushel of cones collected from each. Seed was extracted at the Northern Rocky Mountain Forest Experiment Station at room temperatures, never higher than 80° or 90° F., and duplicate tests of 500 seed each were made in sand flats in the experiment station greenhouse. The results are given in Table I.

TABLE I.—*Germination of duplicate samples of 500 seeds each from girdled hemlock trees*

Progress of germination	Number of days	Percentage of germination of seeds from—					
		Notch-girdled trees			Peeled trees		
		Test A	Test B	Average	Test A	Test B	Average
Beginning	19	11.2	5.0	8.1	0.8	0.2	0.5
Height	28	74.8	51.8	63.3	31.4	16.2	23.8
Last active	38	87.0	68.0	77.5	51.4	44.6	48.0
End	50	89.2	73.8	81.5	53.4	46.0	49.7

It is apparent from these tests that winter girdling, according to either the stripping or notching method, does not prevent the production of a crop of fertile hemlock seed the fall after girdling. Whether the girdling actually causes an increase in the amount of seed produced by these trees above the amount they would have borne if they had not been girdled is not definitely indicated. The crops from girdled trees appeared to be uniformly very heavy, but this occurred in an exceptionally good seed year for all species in the white pine region.

That the percentage of fertility of the seed from the tree girdled by stripping was but little more than half that shown by the tree girdled by notching, does not lead to any definite conclusion, for individual trees are known to vary widely in the quality of seed produced, regardless of external influences. The seed produced by the tree that had been stripped was sufficiently fertile (50 per cent) to restock the area effectively, so it is not possible to consider stripping the bark a better method than notching on the basis of this data. The other object of the girdling, the reduction of shade, was also defeated by the fact that the trees remained green all summer, at the time when white pine and larch seedlings were germinating and establishing themselves.

Thus during the first year after cutting, at the time when surface conditions for germination of desirable intolerant species are most favorable, the girdled hemlocks not only produced heavy crops of fertile seed, which on one particular area resulted in a stand of some 90,000 hemlock seedlings per acre by actual count, but also hindered by their shade the germination and establishment of intolerant white pine and larch on a considerable portion of the area.

Particular note was taken of the effect during the second season after these girdling experiments. A great majority of the completely girdled trees showed a very unthrifty appearance, and this was true, apparently to an equal degree, of the trees girdled by either the notch or stripping method. The girdling on the stripped trees was, however, more often incomplete on account of the strips of inner bark left, and consequently the notch girdling was effective in a larger percentage of cases of the trees girdled.

On several of the sale areas where the girdling had been done, the brush had been burned in piles that autumn. It was noticed that this brush burning had been very effective in killing both large trees

of the undesirable species, and thickets of saplings and advanced growth. The effect of this burning had been immediate, causing the crowns to die and the leaves to fall off within a short time after the burning. The effect of heat and fire on saplings and reproduction was surprisingly great, whole thickets being completely killed by small fires which actually came in contact with only a small proportion of the trees. On the other hand, the trees which had been girdled but not burned were just beginning to die and lose their leaves at the end of the second growing season after girdling, and after they had produced one good seed crop. The much greater effectiveness and value of the brush burning, compared to girdling, were very apparent. From this it is thought that girdling had best be done after brush has been burned, in order to supplement the burning and reduce the cost of cleaning up the area.

For the purpose of finding a more effective way of killing the white fir and hemlock by girdling or other means, an experiment was later installed which was planned to take into account available information on the physiological functions of trees.

In the light of present knowledge of these functions it is easy to account for the fact that the two girdled hemlock trees from which seed was collected remained green throughout the summer following the girdling and produced heavy crops of seed. The girdling did not prevent the movement of water, soluble salts, and stored food from the roots through the sapwood to the leaves. Nourished thus with this water and stored food, it was possible for the foliage to continue photosynthetic manufacture of new food during the summer after girdling. Since most or all of the surplus food could not return to the roots for storage, because of the girdling of the inner bark, it remained in the trunk and crown and was doubtless available for the production of a heavy crop of seed.

There is reason to think, furthermore, that girdling does not entirely prevent the movement of surplus food from the foliage to the roots. This theory is advanced by S. B. Elliott,[2] who cites instances of white pines that have lived from 5 to 22 years after girdling.

EXPERIMENTS IN GIRDLING AND POISONING

As winter girdling had not proved entirely successful, it was decided to try girdling at different times during the growing season, when, in accordance with the theory of food movement, there would be a minimum of stored food in the sapwood available for the production of seed and continued growth. Accordingly, it was decided to test 10 trees each of white fir and hemlock on each of the following dates:

May 15 (beginning of active growth).
June 15 (height of active growth).
July 15 (slackening of growth).
August 15 (height of storing of surplus food).
September 15 (slackening of vegetative activity at end of growing season).

[2] ELLIOTT, S. B. THE PROBLEM OF FOOD MOVEMENT IN TREES. Forestry Quart. 12: 559–561. 1914.

One hundred trees, varying in size from 7 to 28 inches in diameter, and in crown class from intermediate to dominant, were selected in an overmature white pine stand on the Priest River experimental forest. The average diameter of the 50 hemlocks was 15 inches at breast height, while that of the 50 white firs was 13 inches. Heights varied from 70 to 115 feet, averaging 90 feet for both species. The range in age was from 100 to 200 years or more.

Four different methods of straight girdling were used on each date, as follows:

(1) Bark stripped off at base for a height of from 2 to 3 feet.

(2) Trunk girdled with an axe notch cut in the sapwood to a depth of from 1 to 2 inches.

(3) Bark stripped off at base for a distance of from 2 to 3 feet and an axe notch cut into the sapwood to a depth of from 1 to 2 inches, at about the middle of the stripped belt.

(4) Notch from 2 to 3 inches deep, cut through sapwood with a saw and chipped out with axe.

In addition to the girdling, poisoning experiments were begun on a small scale. The following three methods of introducing the poisonous material were used:

(1) Two short notches, 6 to 7 inches long and 2 to 3 inches deep, with their inner edges an inch or two lower than their outer edges were chopped into the sapwood on opposite sides of the tree. They were then filled with a saturated solution of either copper sulphate, copper acetate, or zinc chloride.

(2) Four auger holes, 2 inches in diameter and 3 inches deep, were bored at the base of the tree on opposite sides, and chemicals were put in the holes, as saturated solutions (in the May treatments) or in dry form with water added (as in the June and July treatments). The chemicals used in this treatment were copper sulphate, copper acetate, zinc chloride, copper carbonate, lye, and sodium chloride.

(3) A notch 1 to 2 inches deep was cut completely around the tree, as in method 2 for girdling only, and two 2-inch auger holes were bored at the base of the tree on opposite sides. These holes were filled with the same chemicals used in the foregoing test (2), a saturated solution being used in the May treatments and dry chemicals [3] with water added in June and July.

EFFECT ON FOLIAGE

Table II gives a summary of the effect of different treatments on the appearance of the foliage, in every instance where such an effect was noticeable at the end of the season.

[3] The reason for trying the dry chemicals was to see if the gradual dissolving and absorption into the sap would be any more effective than the rapid absorption of solution.

TABLE II.—*Effect of girdling and poisoning treatments during period of growth, as observed at end of season*

Treatment	Species	Date treated	Effect on tree		
			Unthrifty [a]	One-fourth dead [b]	Dead
GIRDLING					
Bark stripped off at base for distance of 24 to 40 inches.	Hemlock	May 15	1		
	do	June 15	1		
	White fir	do		1	
	do	Aug. 15		1	
Girdled with axe notch 1 to 2 inches deep	Hemlock	Sept. 15	1		
	do	May 15	1		
	do	June 15	1		
	White fir	do			1
	do	do		1	
	do	Aug. 15		1	
Barking and girdling combined	do	do	1		
Notch sawed and chipped 1 to 2 inches deep	Hemlock	June 15			1
POISONING					
Notches 6 to 7 inches long filled with saturated solution copper sulphate.	do	May 15	1		
Four 3-inch auger holes filled with saturated solution copper sulphate.	do	do	1		
Notches filled with saturated solution copper acetate.	do	do	1		
Girdling notch and two auger holes filled with dry lye.	White fir	July 17			1
No treatment control	Hemlock		1		
Total	Hemlock		9		1
	White fir		1	4	2

[a] Distinct yellow-green color of foliage.
[b] One-fourth of inner leaves dead.
[c] Almost dead or dead after Sept. 15.

The damaging influence of the treatments at the end of the first season was, on the whole, negligible. Of 85 trees treated (omitting 8 untreated control trees included in the series, and 7 trees blown down early in the season), only 16 showed any apparent effect whatever. Of these, only 3 had died (1 hemlock and 2 white firs). The other 13 trees (8 hemlocks and 5 white firs) appeared to be distinctly more yellow-green in color than their untreated neighbors, and showed more than the ordinary number of dead leaves. Not much importance can be attached, however, to this yellow-green appearance, as it may easily have been due to slight differences in exposure, soil, natural vigor of the tree, or other factors. The dying of inner leaves may have been just a natural shedding, as indicated by the fact that among the unthrifty trees was an untreated control tree. It is interesting to note, however, that the three trees which died were all girdled with a notch, two of them being axe notches and one a sawed notch. The percentage of the total basal area of the tree occupied by the notch ranged from 13.5 to 65.3, averaging 39.8 per cent for hemlock and 44 per cent for white fir. There seemed to be no relation between the death of the three dead trees and this percentage, the notches on the two white firs being below average and that on the hemlock somewhat above.

Chemicals had been used in only four cases out of the 16. In two cases hemlock trees which showed a yellow-green cast to the foliage had been treated in open-chopped notches—in one instance with a

saturated solution of copper sulphate, and in the other with a saturated solution of copper acetate. In another case a white fir which had been both completely girdled with an axe notch and treated with dry lye placed in two auger holes, died toward the end of the season. The fourth case, a hemlock, showed signs of unthriftiness after a saturated solution of copper sulphate had been placed in four 3-inch auger holes bored in the trunk.

Since the copper sulphate, copper acetate, and lye showed no apparent effect in the several other cases in which they were used, it is probable that they had little or no influence in these treatments. It was noted in the treatments where dry chemicals were used that the more easily soluble materials—zinc chloride, lye, and sodium chloride—had been entirely absorbed, while the copper sulphate, acetate, and carbonate, were only partially dissolved, the residue amounting to from one-fourth to one-half or more of the amount of chemicals used.

One indication of the effect of girdling, it was thought, would be the extent to which the sapwood had dried out, since this would show the degree of restriction of the circulation of water and stored food upward from the roots to the crown. Accordingly, the depth of drying at the last observation, on November 17, was determined for a majority of the girdled trees. Measurements were taken at the center of the stripped portions, and inward from the inner edge of the notches.

Considerable irregularity was noticed in different trees and at different points on the same tree. The dryness on the stripped trees extended from 0.1 to 1.1 inch with an average of 0.4 inch. In the case of the notched trees, the drying extended 0.75 inch to 2 inches in from the innermost point of notch, with an average of 1.1 inch. The depth of the notches ranged from 1 to 3.75 inches, averaging 1.7 inches. The average depth of drying on the notched trees was 2.8 inches compared to 0.4 inch on the stripped trees.

Taking 15 inches as the average diameter at the point of girdling, the average reduction in the amount of wood available for the transmission of water and sap was 60 per cent of the cross-sectional area of the trunk on the notched trees compared to 5 per cent on the stripped trees. This difference indicates that the notching causes a twelve times greater restriction in the passage of water and food than does the stripping, and probably accounts for the fact that the only trees which died were those which had been notched.

Careful observations were made in the fall with field glasses, but no new cones were seen on any of the trees, although several cones left from the preceding year's crop were noticed on some of the hemlocks. This failure of the seed crop can not be attributed to the treatment, however, since there was an almost complete failure of the seed crop of all species in the region that year, due, probably, to severe frosts late in June.

TIME REQUIRED FOR TREATMENTS

The time required for each treatment was recorded. Table III gives the average figures obtained:

TABLE III.—*Time consumed in girdling experiments*

Kind of treatment	Western hemlock		White fir		Both species	
	Average d. b. h. [a]	Time per tree (1 man)	Average d. b. h.	Time per tree (1 man)	Average d. b. h.	Time per tree (1 man)
	Inches	*Minutes*	*Inches*	*Minutes*	*Inches*	*Minutes*
Bark stripped off	18	8	13	7	15	7
Girdled with ax notch	15	7	13	4	14	5
Stripped and girdled with notch	14	9	12	11	13	10
Girdled with sawed notch	14	11	11	11	12	11
2 to 4 chopped notches 7 inches long filled with chemical	12	8	13	5	12	6
4 auger holes 3 inches deep filled with chemical	14	10	13	9	13	9
Girdled with ax notch, 2 auger holes filled with chemical	16	10	12	8	14	9

[a] Diameter at breast height.

It is apparent from this record that the method of girdling with and ax notch was distinctly faster than any of the other methods, requiring an average of 5 minutes of one man's time to a tree compared to 7 minutes for the stripping method, 10 minutes for the combined stripping and notch methods, and 11 minutes for the sawed-notch method.

CONCLUSIONS

No definite conclusions can be drawn from this work as yet because of the small number of instances showing effects of these treatments, and also owing to the possibility of developing further and more satisfactory methods of eliminating undesirable hemlock and fir. It seems probable, however, that girdling with an ax notch is the most desirable method, both in respect to cost and effectiveness. Also it seems that girdling in the spring and early summer (April, May, and June) is more effective than girdling during the late summer or winter.

So far, burning as a means of getting rid of these trees has not been thoroughly investigated, and it is planned to carry out further work along this line in order to determine just how far elimination can be accomplished when the slash from timber sales is burned.

COMPARATIVE SUSCEPTIBILITY OF ONION VARIETIES AND OF SPECIES OF ALLIUM TO UROCYSTIS CEPULAE[1]

By P. J. ANDERSON

Formerly Research Professor of Botany, Massachusetts Agricultural Experiment Station

INTRODUCTION

Most pathogenic fungi show a distinct variation in their ability to infect different varieties of their host plant. Such a difference in varietal susceptibility is usually the starting point for the breeding or selection of resistant varieties, which is the most effectual method for the control of plant diseases. With respect to onion smut, however, it has never been shown that there is any difference in the susceptibility of the numerous varieties under cultivation; in fact, no very comprehensive variety tests with this objective in view have been conducted. Walker and Jones (*12*)[2] tested a few varieties, and Whitehead (*13, p. 449*) in England tested 21 varieties of onions and 11 varieties of leeks (*Allium porrum*), but none showed resistance. Nevertheless, since there were numerous other varieties which had not been tested, so far as was known to the writer, it appeared worth while to collect seed of as many varieties as could be obtained and test them comparatively in the hope that some of them might show a degree of resistance which should warrant crossing and selection work.

The genus Allium, to which the cultivated onion belongs, is a large genus of some 250 species which are widely distributed over the earth. *Urocystis cepulae* was first reported on *Allium cepa* (*4, 8*) in 1857, in eastern Massachusetts. As early as 1881 (*7*) it was also reported on *A. porrum* (leek), in France. Cotton (*3*) reported it as more serious on this species than on onion in England. Clinton (*2, p. 451*) reported it as occurring on *A. nevadense* in the far West of the United States. Quite recently Zillig (*14, p. 57*) has also reported infection of *A. fistulosum* L., *A. globosum* Red., and *A. odorum* L. Thus this fungus has been found on five species of the genus besides *A. cepa*.[3]

A more thorough knowledge of the extent of its host range is desirable for at least three reasons:

(1) It might give us a clue to the origin of onion smut. This disease seems to have been noticed for the first time on onions in New England about the middle of the last century. Where it came from and where it had passed the preceding centuries, nobody seems to know. There is the strong probability that it came from some native American susceptible Allium; yet, with the exception of

[1] Received for publication October 22, 1924; issued, September, 1925.

[2] Reference is made by number (italic) to "Literature cited," p. 286.

[3] After this paper had been forwarded by the writer for publication, a letter was received from Doctor Zillig stating that he had also been able to infect *A. cyaneum* Reg., *A. flavum* L., *A. huteri* Sund., *A. hymenorrhizum* Ledeb., and *A. obliquum* L.

Allium nevadense in the far West, no American Allium is known to be susceptible, so far as the writer is aware. If it were found that some one of our New England Alliums is subject to this disease, then an explanation of its origin would be easy.

(2) It would help settle the question of a possible relationship between *Urocystis cepulae* and some of the other species of Urocystis which occur on other species of Allium.

(3) In a genus as large as Allium, it is probable that there are some resistant species which may be crossed with *A. cepa* and that from the hybrid progeny of which a desirable smut-resistant onion might be developed. The initial step is to determine how resistant the different species are, if they are resistant at all.

EXPERIMENTS WITH ONION VARIETIES

From the catalogues of American seed houses, 25 onion varieties which seemed to be distinct were selected. Seed of 29 European varieties were obtained from H. Zillig, of the Biologische Reichsanstalt für Land-und Forstwirtschaft, at Trier, Germany, who also was investigating the same phase of onion smut and who kindly sent seed of each of the varieties which he was trying. Three hundred seeds of each of the 54 varieties thus obtained were planted April 25, 1924, in soil which was known to be very heavily infested with smut. When the seedlings came up there was seen to be considerable variation in the percentage of germination. Smut had appeared on all of the varieties after three weeks. All plants, irrespective of infection, were counted on May 30 to determine the percentage of germination, and at that time smut infection was estimated at about 75 per cent on all varieties and none offered evidence of being strikingly more resistant than others. The smutted ones had already begun to die and continued to drop off throughout the summer. On August 11, all the remaining plants were pulled and counted, and the percentage of infection was determined by comparing the number of healthy plants at that time with the number which had germinated on May 30.

The results are presented in Table I. They do not indicate that there is any important degree of resistance in any of the 54 varieties tried. Neither does the theory of some onion growers, that the red varieties are more resistant, find any support in these results. The pigmentation factor shows no relation to the resistance factor. There are, however, still other varieties which have not been tested, and it is possible that some more resistant variety may be found among them. It is therefore important that this line of work should be continued.

TABLE I.—*Susceptibility test of varieties of Allium cepa*

Name of variety	Source of seed	Number of plants May 30	Healthy plants Aug. 11	Percentage of infection
American varieties:				
Australian Brown	Livingston Seed Co	195	58	70
Bermuda Yellow	F. H. Woodruff & Sons	210	33	84
Crystal white wax	do	177	21	88
Early Barletta	do	163	39	76
Early Neapolitan Marzajola	D. M. Ferry & Co	0	0	----
Early Yellow Cracker	Fottler-Fiske-Rawson Co	73	2	98
Extra Early Red Flat	F. H. Woodworth & Sons	176	17	90
Extra Early Yellow	do	152	12	92
Giant White Italian Tripoli	D. M. Ferry & Co	0	0	----
Isbell's Early White Sugar Ball	S. M. Isbell & Co	52	0	100
Isbell's Evergood Red Globe	do	109	10	90
Large Red Wethersfield	F. H. Woodworth & Sons	95	10	89
Mammoth Silver King	Livingston Seed Co	78	12	84
Ohio Yellow Globe	F. H. Woodworth & Sons	66	5	93
Prizetaker	do	136	28	78
Red Italian Tripoli	Fottler-Fiske-Rawson Co	17	3	80
Southport Red Globe	F. H. Woodworth & Sons	83	15	81
Southport White Globe	do	132	14	89
Southport Yellow Globe	do	182	21	88
White Adriatic Barletta	Fottler-Fiske-Rawson Co	56	1	98
White Bunch	Alexander Forbes & Co	125	26	79
White Portugal	F. H. Woodworth & Sons	94	5	94
Yellow Danvers Flat	do	150	20	86
Yellow Dutch or Strassburg	do	136	12	91
Yellow Globe Danvers	do	166	9	95
European varieties:				
Blassrote hollandische	H. Zillig	119	10	91
Blut rote Ulmer	do	147	36	75
Bornaer Riesen	do	64	10	85
Bronzekugel	do	7	0	100
Braunschweiger schwarzrote	do	15	2	86
Calbenser Lokalsorte	do	71	22	68
Eisenkopf	do	103	17	83
Erfurter blassrote	do	26	4	84
Franzoschische oder spanische weisse	do	155	29	81
Gelbe Birn	do	85	18	78
Hollandische blutrote plattrunde harte.	do	119	16	87
Hollandische gelbe plattrunde harte	do	96	10	89
Hollandische silberweisse plattrunde harte.	do	90	6	93
Liegnitzer Lokal-Sorte	do	145	16	88
Runde gelbe Stuttgarter Riesen	do	38	6	84
Silberweisse Konigin	do	116	12	89
Silberweisse plattrunde fruhe	do	127	11	91
Strohgelbe (hellgelbe) plattrunde	do	33	1	97
Tripoli Rocca	do	118	17	85
Vertus	do	58	8	86
Weisse fruheste Fruhlings-Zwiebel	do	163	10	93
Zittauer Riesen—				
Blutrote runde	do	18	0	100
Gelbe runde	do	7	1	85
Schwefelgelbe	do	112	19	83
Silberweisse runde	do	161	20	87
Blassrote plattrunde harte Erfurter	do	162	4	97
Dunkelrote plattrunde harte Braunschweiger.	do	191	8	96
Grosse gelbe runde Zittauer Riesen	do	125	2	98
Hollandische gelbe	do	110	0	100

EXPERIMENTS WITH OTHER SPECIES OF ALLIUM

During the last three years the writer has been attempting to determine the susceptibility of all species of Allium of which viable seed could be obtained. The seeds were planted in soil known to be heavily infested with the smut fungus and when the seedlings came up the percentage and severity of infection were recorded weekly until the plants were well grown and there seemed no more probability of new infections. Progress of the investigation has been slow on account of the difficulty of securing viable seed. Letters sent to the American seed houses and botanical gardens where one might expect

to find seed of wild species netted two or three species which did not germinate. Somewhat better success was had in Europe and most of the seed which the writer has obtained came from Kew Gardens, Haage & Schmidt, of Erfurt, Germany, H. Zillig, of Trier, Germany, and Vilmorin–Andrieux & Co., at Paris. Only last summer did the writer succeed in getting seed (or bulblets) of all the three native New England species of Allium. Allium seed seems to lose its vitality very rapidly, and seed more than a year or two old frequently can not be made to germinate. The writer thinks it is possible, of course, that he has not been able always to provide in the plots the special conditions which may be necessary to bring some species to germination. Altogether seed of about 100 species has been tried and the seed of only 39 have germinated.

In the course of this work it has been observed that there are gradations in the severity of infection. On this basis all the species may be divided into the following classes:

Class 1. Most susceptible class. Sori first appear in the cotyledons and may of the plants die at this time. Those which survive may slough off the disease, or lesions may appear in the successive true leaves, ending in the final death of the plant before it forms seeds.

Class 2. Less susceptible than class 1. Smut sori occur only in the cotyledons, and many plants die in this stage, but if they survive no lesions appear in the true leaves.

Class 3. Species barely susceptible in that smut sori may be found in the cotyledons, but the plants are not killed in this or later stages and no sori appear in the true leaves.

Class 4: Entirely resistant species in which smut lesions have not been observed in any of the parts.

In infested soil from the same field three different plantings were made. Nineteen species were planted May 1, 1923 in flats kept out of doors; 40 species in a greenhouse bench on October 11, 1923; and 64 species in an outdoor bed on April 25, 1924. Some species were tested in all three plantings, some in two, and some in only one, depending on the supply of seed and the need of further testing. In cases where fewer than 100 plants came up this fact is mentioned in the following notes on the individual species.

CLASS 1

Allium Cepa L., to which all our varieties of cultivated onions belong, is typical of Class 1. All infection seems to take place through the cotyledon. A large percentage of infected plants die in this stage. If no sori occur in the cotyledon, none will be found in the true leaves which follow. Sometimes, even when the cotyledon is affected, the fungus fails to gain entrance into the growing point and no lesions will be found in the following leaves. In the latter case, the bulb and seed are developed normally. More often, however, the parasite gets into the growing point and each successive leaf will have spore sori, in which case the leaves are short, distorted, and brittle, and the dwarfed plants die in various stages of development throughout the summer. Some of them form bulbs as large as an inch in diameter with longitudinal black spore pustules in the outer scales, but they usually rot before they are harvested. It is doubtful whether a plant which has spores in the first leaf ever recovers. In the soil

which was used for the comparative susceptibility experiments, 80 to 100 per cent of the seedlings which appeared died before harvest.

Allium fistulosum L. (Welsh onion). This onion reacts in every way like *A. cepa*, except that the percentage of infection is not so high. In the first planting 50 per cent were infected, 5 per cent in the second, and 25 per cent in the third. Infected plants succumbed in various stages of development. This species is a robust, rapid grower, and lives through the winter out of doors. There was no difficulty in getting a high percentage of the seed to germinate. Zillig also found this species susceptible.

Allium ascalonicum L. (Shallot). Only one planting was made, and about 100 strong plants started growth. Ninety per cent had smut in the cotyledons, and those which did not die in this stage had smut in the successive leaves and died throughout the summer, just as did *A. cepa*.

Allium hookeri Thwaites. In the first planting 10 per cent were infected. In the second planting 95 per cent were infected, and the majority of the plants died at an early stage. Sori were found in the fourth and fifth leaves, but no smutted plant reached maturity. This species seemed just as susceptible as *A. cepa*.

Allium libani Boiss. In the first planting 50 per cent had smut, and in the second 95 per cent of the cotyledons had it. A large percentage of the plants died in that stage. Only a few showed smut in the later stages, and none of the diseased ones developed to maturity.

Allium senescens var. *giganteum*. About 100 plants started growth from the one sowing made. Smut lesions were in the cotyledons of almost all of them, and many died in this stage. Lesions also occurred in the early true leaves. By the middle of the summer only 15 plants had survived, but they were free from smut. This appears to be a very susceptible variety and is remarkably different in this respect from the rest of the species (if, indeed, this is only a variety of the form listed below as *A. senescens*, the seed of which was received under the name of *A. fallax*).

Allium huteri Sund. Although a large number of plants came up, they all died before the first leaf was fully developed, and all were found to be full of smut lesions. This species appears to be so susceptible that it never gets much past the cotyledon stage in an infested soil.

Allium flavum L. There was 95 per cent infection in the cotyledons of the hundreds of plants of this species, and a heavy mortality at this stage. Sori were found in young leaves of some which continued to live, but no smutted plants were found later in the summer. The mortality seems to be all in the young stages.

Allium schoenoprasum L. (chives), is much more resistant than *A. cepa*. Infection occurred in only about 2 to 5 per cent of the cotyledons. Later in the summer sori were found in the leaves. Affected plants did not divide and produce tufts of shoots as did the healthy ones. All infected ones died before the end of the summer.

Allium schoenoprasum var. *sibiricum* L. Only two plants came up when this seed was planted. One of the two had smut sori on it.

Allium nutans L. This is a very susceptible species. Two plantings were made, and some hundreds of seedlings came up. Ninety

per cent of these seedlings had smut in the cotyledon, and most of them died in this stage, but a few continued to develop and had smut sori in the true leaves up to the fourth or fifth leaf, but no sori were found on any plant that was more mature. This species almost falls in class 2.

Allium neapolitanum Cyr. Not more than 1 per cent of the hundreds of seedlings which grew from one planting showed smut. Lesions were found, however, in the first true leaf. Hence this species is placed in class 1, although it appears to be very resistant.

CLASS 2

Allium porrum L. (leek). In England this species is said to be attacked more severely than *A. cepa* (*3, p. 170*). Zillig also was able to infect it, but neither he nor Cotton described the effect of the disease on the plants. Malbranche (*7*) stated in 1881 that 20 per cent of the *porrettes* (young leeks) in certain gardens in Rouen were attacked, and that the disease had established itself in the blades and especially the bases of the leaves. The writer made two plantings and had no difficulty in raising hundreds of plants to maturity. The first planting showed 15 per cent of infection in the cotyledons, and the second 10 per cent, but only a very few of the plants died. The writer has searched in vain for sori in the true leaves of the plants. The damage caused by the parasite here is negligible. These observations are surely at variance with those of Malbranche and other European investigators. Apparently the disease is more serious on leeks in Europe than in America.

Allium angulosum L. From the one planting made several hundred seedlings were obtained. Ninety-five per cent of the cotyledons had smut, and more than half of the plants died in this stage. Those which survived, however, showed no trace of smut in the leaves. This seems to be an extremely susceptible species, but the mortality is all in the early cotyledon stage.

Allium nigrum L. Considerable interest is attached to this species because of its relation to *A. magicum*, on which Passerini collected a smut which he named *Urocystis magica*. (In the past there has been considerable discussion as to whether *U. magica* is identical with *U. cepulae*. If it is, the onion smut fungus may well be of European origin. *A. magicum* is either a variety or a synonym of *A. nigrum*, according to the authority one wishes to follow.) One sowing was made, but the germination was poor; only about 30 seedlings grew. One-third of these had smut in the cotyledon, but none was observed in the later leaves, although all the plants were grown to full maturity and produced blossoms and seeds. The original specimens of *U. magica*, collected by Passerini and examined by the writer in the Harvard herbarium, were on very large leaves. The fact that the large leaves of *A. nigrum* have not been observed to become infected would indicate that the fungus Passerini collected and named was not identical with *U. cepulae*. More thorough infection tests should be made, however, the writer believes.

Allium pulchellum G. Don. Three plants came up and died of smut in the cotyledon stage.

Allium sikkimense Baker. Only two plants came up. One showed smut lesions and soon died.

CLASS 3

Allium volhynicum Bess. Two plantings were made. Ten per cent of those of the first planting had smut in the cotyledons, and 50 per cent of the second planting had it. No lesions were observed in the true leaves.

Allium polyphyllum Kar. and Kir. Two plantings were made, but the germination was poor. Only six plants were obtained. Smut lesions occurred in the cotyledons of half of them, and some died later, but the writer is not sure that the smut killed them. Further tests might place this species in class 1 or 2.

Allium scorodoprasum L. Same general condition as for the preceding. Only a few plants came up, but some of them had lesions in the cotyledons.

Allium obliquum L. Of several hundred plants which came up 90 per cent had infected cotyledons, but the later leaves showed no sori.

Allium senescens L. (*A. fallax* Schult). Out of about 150 seedlings from the first planting, smut lesions were found in only five cotyledons. In a second planting, 2 per cent of the cotyledons had smut. No lesions were observed in any of the leaves, and it was not observed that any of those which had smut in the cotyledons died. Apparently here is a very resistant species, but not immune.

Allium ampeloprasum L. Only a few seedlings were obtained. About half of them had sori in the cotyledons.

Allium darwasicum Reg. Only 14 seedlings resulted from the one planting made. Three or four of them had smut lesions in the cotyledons.

Allium montanum Sib. and Sm. Twenty seedlings grew from the one planting. Two of them had infection in the cotyledons, but no infected ones were observed later.

Allium paradoxum Don. Of the three plants which came up, one had cotyledons lesions, but no disease was observed in later stages.

Allium odorum L. Three plantings with seed from as many different sources were made, and hundreds of plants were grown. No smut was observed in any of them. The results were at variance with those reported by Zillig, who was able to infect this species. Subsequent to the third planting, Zillig kindly sent some seed which he obtained from the Botanic Gardens in Munchen. When plants grew from these, about 10 per cent had lesions in the cotyledons, this suggesting the possibility that in this species we may have varieties or strains which differ in their susceptibility to the smut organism.

Allium recurvatum Rydb. Of the 50 plants which came up, one-half had smut in the cotyledons, but no lesions were observed later on the true leaves. Although many of these plants died in a young stage, it was not evident that the smut was the cause.

CLASS 4

Allium oreoprasum Shr. Two plantings were made, and hundreds of seedlings grew, but no smut sori appeared on any of the parts at any time. As far as these trials showed, this species is perfectly immune.

Allium moly L. Only one planting with this species was made. The germination was poor, and only a dozen plants were obtained;

none of them showed infection in any stage. Although the writer has had these plants growing for two years, none of them has produced seed.

Allium heldreichii Boiss. Only three plants came up, and none of them was infected.

Allium macranthum Baker. About 50 plants were obtained from the single planting. No lesion was found at any time on any of them.

Allium subhirsutum L. About 100 plants came up. No smut was observed in them at any stage.

Allium roseum L. A very few plants came up and soon died, but no smut was observed. The writer is of the opinion that further tests should be made with this species.

EXPERIMENTS WITH SPECIES WHICH REPRODUCE BY BULBLETS

It has long been known that when onions are started from sets (small bulbs) they are entirely free from smut. In a previous publication, the writer has shown that all infection of onions takes place through the cotyledons (*1*). As there are no cotyledons when a plant starts from a bulblet, it is easy to understand why onions from sets are immune. There are many species of Allium which never produce seeds but reproduce by small bulblets which form in dense heads in a position much like the seed clusters of seed-bearing species. There are other species which produce both bulblets and seeds, and all gradations may be found between these two methods of reproduction. The most common native wild onion in New England (*Allium canadense*) reproduces by bulblets only. Although the writer has found plants which were producing flowers and seemed to be setting seed, he has never found that the seed matured in this section of the country. Bulblets of this species have been planted and many plants grown to maturity, but no smut has been found at any time. The results were the same using bulblets of *A. roseum*. The writer has not been able to obtain viable bulblets of other species, but there is every reason to believe that the results would not be different. Probably none of the bulblet-producing species are ever infected. In view of the widespread tendency toward the bulbiferous habit throughout the genus *Allium*, it is an interesting speculation as to the influence which the smut fungus may have had in eliminating the seminiferous strains and forcing by natural selection the development of bulbiferous species.

EXPERIMENTS WITH THE WINTERBECK ONION

Some seed labeled "Winterbeck Zwiebel" were received from Haag & Schmidt, Erfurt, Germany. The writer is not sure whether this should be considered a distinct species or only a variety of *Allium cepa*. It comes up more quickly than the common onion, and grows more rapidly, but does not develop a large bulb, and the bulb divides more readily, somewhat with the same general habit as the Welsh onion and the multiplier (or Egyptian) onion. Unlike the latter, however, it does not produce a head of bulblets but reproduces entirely by seeds which form in heads, as in the case of the common onion. It is a stronger grower and more hardy than the common onion, less susceptible to damping-off and thrips. It is

regarded as all but immune to smut. Not more than 2 or 3 per cent showed smut in the cotyledons, and no smut was seen in any later stage. In fact, smut is an entirely negligible factor. This seems to be the logical parent species for the breeding of a smut-resistant onion by crossing.

THE RELATION OF UROCYSTIS CEPULÆ TO OTHER SPECIES OF UROCYSTIS ON ALLIUM

As far as the writer is aware, no Urocystis other than *Urocystis cepulæ* has ever been found on the cultivated onion. Investigators who have found the disease agree as to the remarkable constancy of characters of this species so that not even a variety has been suggested. On other species of Allium, however, four other species of Urocystis have been found, and from the first description of the species up to the present mycologists and pathologists have discussed the relationship of these other species of Urocystis to *U. cepulae* without reaching any agreement. Some have considered all five forms as distinct species, some would unite them all under one species, i. e., regard the onion smut fungus as a variety of or identical with some of the others. Various other combinations are proposed. Not until all of these species have been cultured and studied and cross inoculations made on all host species concerned will this question be definitely settled; but it is hoped that the inoculation experiments just described and supplemented by examination of exsiccati of the pathogenes may contribute something to its solution. Let us examine briefly the other four species.

Urocystis magica Pass. was named by Passerini from specimens which he collected on *Allium magicum* at Parma, Italy, in 1875, and distributed in Rabenhorst's Fungi europaei as No. 2100 and in von Thuemen's Mycotheca Universalis as No. 223. The writer knows of no record that the fungus has ever been collected again on that host in Italy or elsewhere. All subsequent literature merely refers to the original description or consists of observations on studies of Passerini's original collection. It is remarkable that a fungus which was so abundant that it could be furnished in plentiful supply for distribution in two exsiccati should never have been collected again. The writer had occasion to study specimens of both of these exsiccati in the Harvard University herbarium. The long (some of them more than an inch) raised sori were on broad flat leaves. *A. magicum* is considered by most phanerogamic authorities as a synonym or a variety of *A. nigrum* L. This latter species, however, does not have flat leaves, but linear, terete leaves. Apparently, then, the plant on which Passerini collected the parasite was not *A. nigrum* but some other species. The spore balls contained a single spherical, or short, oval, brown central cell surrounded by hemispherical accessory cells. Only rarely did the writer find two fertile cells in a spore ball, and these were not attached by their surfaces, but were held together because surrounded by a common layer of sterile cells. In shape, color, and arrangement of all parts these spores could not be distinguished from those of *U. cepulae*. There is, however, a constant difference in size; the spores of *U. magica* were larger. Fifty spores from each species were measured under identically the same conditions. The central cell of *U. magica*, mounted in lactophenol, measured 14.27 by 15.55 μ as compared with 11.04 by 11.75 μ for *U.*

cepulae. The diameter of the entire spore ball of *U. magica* was 22.19 μ, while that of *U. cepulae* was 16.15 μ. All dimensions are somewhat greater when the spores are measured in a potassium hydroxide solution or when free. When the spores have been kept in a dry state for a long time, the accessory cells collapse but become distended again when treated with a weak solution of potassium hydroxide. Farlow (*5, p. 114*) considered *U. magica* as identical with *U. cepulae*. Schroeter and Winter (Die Pilze, p. 121, 1884) agree with Farlow and unite all the forms discussed here under *Urocystis colchici* Schlecht. Thaxter (*11, p. 144*) and Clinton (*2, p. 451*), on the other hand, point to the differences in the size of the spores as sufficient reason for considering the two species distinct. If by any chance the host plant of *U. magica* was really *A. nigrum*, the fact that the writer was unable to cause infection except on the tiny cotyledons, while in the exsiccati the fungus is on large leaves, seems to the writer another argument against the identity of the two. The constant difference in the size of the spores as observed by all investigators, and the difference in host plants, seem to be sufficient reasons for regarding *U. cepulae* as a species distinct from *U. magica*.

Another species of Urocystis has been reported on *Allium rotundum* in Europe by various investigators, and has usually been referred to *U. colchici* Schlecht., a common species there, occurring on a long list of Lilliaceae. Some authors give to the form which occurs on Allium the rank of a variety, *forma Allii* under *U. colchici* because of certain morphological differences. The writer made a study of a specimen of *forma Allii* (Fückel Fungi rhenani No. 2217) collected by Fückel in Austria on *Allium rotundum*. All characters and dimensions were so nearly identical with those of *U. magica*, which were studied at the same time, that, from a morphological standpoint, no reason could be found for considering the two as distinct. These two forms are also doubtfully united by Liro (*6*) in his recent excellent monograph of the genus Tuburcinia.

After the variety on *Allium rotundum* has been removed from *Urocystis colchici*, the species as it occurs on other hosts is easily distinguished from *U. cepulae* because the spore balls commonly are attached in glomerules of 3 to 5 or more, and a single spore ball may have 2 to 3 fertile cells. All dimensions are also larger than those of *U. cepulae*.

Rostrup (*9, p. 153*) found a species of Urocystis on *Allium ascalonicum* in Denmark, in 1890, and referred it to *U. cepulae*. Liro, however (*6, p. 50*), refers it to his newly described species *U. ferruginea* which differs sharply from *U. cepulae* in the red brown color of the spore powder, and in the angular spores attached in groups of six or more. The writer has not had an opportunity to examine this species, but, judging from the description given by Liro, there would seem to be no question as to its distinctness and no occasion for confusing it with *U. cepulae*.

In 1911, Schellenberg (*10, p. 14*) described the species *Urocystis allii* from a specimen collected in Switzerland in 1902 on *Allium subhirsutum* and distributed in von Thuemen's Mycotheca universalis as No. 1219. Although in morphological characters it seems to resemble *U. cepulae* even more closely than does *U. magica*, both Schellenberg and Liro consider it as distinct. As previously mentioned in this paper, *A. subhirsutum* L. was found by the writer to be entirely immune to *U. cepulae*, which is regarded as evidence con-

firming the distinctness of the two species of Urocystis. Another collection of a Urocystis on *A. oleraceum* L. was also referred to this same species by Schellenberg.

ORIGIN OF THE ONION SMUT

What bearing have the facts set forth in this paper on the problem of the origin of onion smut? The writer believes that there are good morphological and biological reasons for considering the smut fungus as a distinct species from any of the Urocystes found on European species of Allium. It should also be pointed out in this connection that even if it should be found that some one of the European species named in recent years was identical with *Urocystis cepulae*, this would not indicate a European origin for the disease. It seems to the writer that it would be much more logical in that case to conclude that the fungus was first on onion and passed from the onion over to the other species where it was collected. In fact, in view of the wide host range which the writer has demonstrated for the onion smut organism, it would seem remarkable if it should not be taken some time by some collector on another species. During the 50 years that it has been in Europe it has had abundant opportunity to spread to other hosts.

In brief, in the writer's opinion, there is not the least evidence that the disease is of Old World origin. On the contrary, there is excellent circumstantial evidence that it did not exist there on any species previous to its discovery in America. Onions have been grown and used by every civilized people in the Old World at least since the building of the pyramids. In all that time onions certainly must have come in contact with every disease which occurred on other species of Allium indigenous to the countries where they grew. Smut is by no means an inconspicuous disease, and if it had been present in the Old World it very likely would have been mentioned before 1872.

Some of the susceptible species mentioned in this paper are native to the New World, and probably further search will add to the list of our indigenous susceptible ones. Some one or more of these was probably the original host. Possibly it was a native of the far West in this country, like the susceptible *Allium nevadense*. For many years after the onion was brought to America smut seems to have been unknown. The writer offers the theory that as the frontier of civilization advanced into the West, taking along with it the onion, the onion came into contact with the organism in its native home, and that the spores could easily have been taken back to New England in the natural course of commerce, with the result that the disease first became prominent in a great intensive onion center, such as eastern Massachusetts was at that time, rather than in the isolated gardens of the pioneers. It may be significant that smut first appeared within the next decade after the California gold rush of 1849.

SUMMARY

None of the 54 varieties of cultivated onions tested showed any considerable resistance to smut.

Out of 39 species of Allium tested, 8 seemed to be immune, and 31 showed varying degrees of susceptibility.

Thirteen species seemed very susceptible, and are in the same class as the common onion.

Smut occurred in the cotyledons of 13 more species, but did not kill the plants or occur in the true leaves.

In five other species it occurred in the cotyledons and killed many of the seedlings in that stage, but did not appear in the true leaves.

Smut did not occur in species which reproduce by bulblets.

On this basis one might expect to find among the 250 known species of Allium at least 150 which are susceptible to smut.

Urocystis cepulae is considered by this writer as distinct from all other species of Urocystis which have been reported on other members of the genus Allium.

All the evidence the writer has indicates that this parasite lived originally on some wild American species and thence passed over to the cultivated onion.

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THE NITROGEN CONSTITUENTS OF CELERY PLANTS IN HEALTH AND DISEASE[1]

By G. H. Coons, *Associate Professor of Botany, Michigan State College*, and L. J. Klotz, *Assistant Professor of Botany, University of New Hampshire*

INTRODUCTION

Studies of the comparative chemical composition of plants in health and disease have, in the main, been few and the results obtained have not been such as to warrant generalizations as to the effects the various pathogenes bring about. Plant pathology has lagged conspicuously behind animal pathology in this regard, for in the latter science chemical pathology is a definite field with a large accumulation of data (*27*),[2] and these facts have permitted certain generalizations. In plant pathology the nature of the disturbance caused by the parasite can only be surmised, as the physiology of plants in disease is largely unknown and awaits a fuller knowledge of the physiology of plants in health.

In the early developments of agricultural chemistry, countless analyses catalogued the composition of plants. But these analyses, largely made to determine relative food value, do not permit the contrasting of plants in health and in disease. There have been observations made upon the development of certain chemical substances in the course of disease—pigments, toxic substances, enzymes, etc.—but these results are fragmentary and restricted in application.

That diseased plants are profoundly changed by the action of pathogenes is common knowledge, and the terms applied, decomposition or decay, indicate the general concept of the nature of the process which has taken place in disease production.

REVIEW OF LITERATURE

Müller-Thurgau (*17, p. 61*) has shown that the must from rotted grapes is poorer in nitrogen than that from sound grapes. The organisms producing the rot were found to use first the easily assimilated stuffs and accordingly the must from rotted berries was found to have nitrogen compounds present in far less extent and in a form less suitable for fungous growth than was the case with must from sound fruit. Similarly, he has also shown (*16*) that with rotted pome fruits, a clear sap almost free from nitrogen is obtained, in marked contrast to what is obtained from sound fruit.

Recently more comprehensive studies on the chemistry of healthy and diseased plants have been published. In a preliminary article, Boncquet (*5*), in discussing the chemistry of the mosaic disease in sugar beets, states, that dentrification sometimes takes place in

[1] Received for publication July 25, 1924; issued September, 1925. The chemical analyses presented in this paper were made by L. J. Klotz (*14*) who has developed the special methods for dealing with the *Septoria apii* material. The work with *Cercospora apii* was conducted in the laboratory of R. C. Huston at the Michigan Agricultural College. The writers wish to acknowledge indebtedness to him for advice. They are also indebted to C. S. Robinson for criticism of the manuscript.

[2] Reference is made by number (italic) to "Literature cited," p. 299.

diseased plant tissues whereby nitrates are converted into nitrite and ammonia. This result led this worker to attribute the disease in question and other diseases of similar nature to denitrifying bacteria.

We are indebted to True and his associates (*23*) for extensive and thorough-going analyses of plants in disease and health made in connection with their studies on spinach mosaic. In this investigation ash content, oxidase reactions, carbohydrate production, and nitrogen metabolism of healthy and blighted spinach were compared. The last named phase of the work was carried out by Jodidi, Kellogg, and True (*23, p. 385–404*), and since this part of the investigation was most extensive and the methods employed have since been the basis of other investigations, it will be considered in some detail. They found, as a result of their comparative analyses, that in diseased spinach there was a lower percentage of total nitrogen and of acid-amide nitrogen than in healthy spinach, and they explained this relation by the assumption that denitrification takes place in those tissues whereby a part of the nitrogen is lost either as elementary nitrogen or in the form of ammonia. They also found a higher proportion of ammoniacal nitrogen in the diseased material than in the corresponding healthy tissues, and the suggestion was made that the reason for this is also to be sought in the processes of denitrification, whereby a part of the nitrites is further reduced to ammonia.

In 1920, Jodidi, Moulton, and Markley (*13*) reported additional analyses along the same lines as those of the previous year, in order to corroborate the early results by observations on spinach taken from various fields and at other seasons. The results of this work seem to agree closely with the results of the earlier work. The presence of nitrites in the diseased plants postulated in the former article was definitely shown.

Jodidi (*12*) in 1920 gave the results of analyses of healthy and diseased cabbage. The diseased cabbage analyzed by him is described as showing a metallic color between the veins, only the spaces nearest to the veins being green. The leaves were brittle and their margins were frequently of a uniform yellow color. Coupled with these signs there was a poorly developed root system and a stunting. It is definitely stated that preliminary experiments to demonstrate the infectious nature of the disease had thus far led to negative results. The work previously mentioned had shown a certain type of the nitrogen distribution in mosaic spinach as contrasted with that in healthy spinach. Jodidi found that the diseased cabbage showed the same relations in the chemical constituents as compared with the healthy cabbage, and he concluded: "Judging from the striking similarity of the analytical evidence presented with regard to the various nitrogenous compounds occurring in healthy and blighted spinach plants, on the one hand, and in normal and diseased cabbage plants on the other, and taking into consideration that spinach blight belongs to the type of mosaic diseases, it appears logical to relate the cabbage disease caused by denitrification to the latter type."

Further contributions to the biochemistry of plants in health and disease have been made by Willaman and Sandstrom (*28*), but in this work the chief emphasis has been placed upon other constituents than the nitrogenous ones.

Reference also should be made to the contribution of Doby (*8*) who studied the biochemical relations of healthy potatoes and potatoes affected with leaf roll and who found lower insoluble protein content in diseased than in healthy tubers, the low protein content being the net result of higher oxidase content of the diseased tubers which lead to a great combustion of the cleavage products of the protein.

EXPERIMENTAL WORK

It was thought desirable to secure for comparison with the work on a mosaic disease which has been cited, analyses from fungous leaf blights where the effect of the parasite brought about a definite circumscribed killing effect upon the leaf tissue. Celery leaves affected with the disease known as "early blight" caused by the fungus *Cercospora apii* Fres. were first used for this purpose. The analyses along with a statement of the methods employed are given below.

For further evidence upon the nature of the changes in the nitrogenous constituents of plants as brought about by fungous attack, material from another celery disease, "leaf spot" caused by *Septoria apii* Chester, was taken. In this disease, the effect upon the tissues is more pronounced than in the "early blight," the fungus commonly producing in its last stages a complete rotting of the affected leaflets and stalks.

The materials chosen, therefore, represent two rather widely differing types of disease, and the analyses given reveal something of the type of decomposition produced by the parasites.

METHODS USED WITH CELERY BLIGHTED BY CERCOSPORA APII FRES

The methods used were in the main those employed by Jodidi and his collaborators (*13*). All the determinations, except water content, were made with diseased and healthy celery of one variety, Easy Bleaching, which was collected from plants of the same age growing in the same garden plot. The work was done entirely on mature leaflets. The lesions on the blighted leaves involved approximately from one-sixth to one-fourth of the leaf area. The leaflets were spread in thin layers on cheesecloth and dried at room temperature for three days and then placed for three days in an electric oven which was maintained at a temperature of 49° to 54° C. The dried materials were next rubbed through a 40-mesh sieve, then mixed to assure more uniform sampling and finally placed in jars and tightly sealed. The dried, powdered leaflets thus made ready for use were of about the fineness of table pepper. The color of the powdered blighted material was an ashen, grayish green, while that of the healthy material was distinctly chlorophyll green.

The total nitrogen was determined by the Kjeldahl and Kjeldahl-Gunning methods, 2 gm. samples of the dry, unpowdered leaves being used in the first method, 2 gm. samples of the more representative powdered materials being used in the second method. It may be said here that in all ammonia distillations 4 per cent boric acid solution was used as the receiving liquid and brom-phenol blue indicator in the titration (*19*).

Nitric nitrogen was estimated by two different methods, F. M. Scales' zinc-copper couple reduction method (*9*), and the Schulze-

Tiemann nitric oxide gas method. (For literature on this and other methods used by Jodidi *et al.* see literature references (*12*, *13*, *23*).) According to Jodidi's procedure, 12 gm. each of healthy and blighted celery were repeatedly extracted and thoroughly washed with 85 per cent alcohol. Milk of lime was then added to the combined extracts and washings, which were then evaporated to dryness in a vacuum oven at low temperature. The residue was taken up with hot water, lead acetate solution added, and the whole filtered, washed, and the combined filtrate and washings made up to 2 liters. In the Scales' method 250 c. c. samples of the extract were placed in 500 c. c. Kjeldahl flasks containing 80 gm. of the Zn-Cu couple coils. Five grams of c. p. sodium chloride and 1 gm. of c. p. magnesium oxide were added and the ammonia from the nitrates thus reduced distilled into 50 c. c. of 4 per cent boric acid. In the gas method 250 c. c. aliquot portions of the same extract were used and the work carried out exactly as described (*1*, *p. 312-315*).

Distillation with magnesia not giving dependable results in the estimation of ammonia nitrogen, a modification of Grafe's method of distillation *in vacuo* at low temperature was employed. Ten-gram samples were placed in 1-liter flasks, treated with 25 c. c. of concentrated sodium chloride solution, 35 c. c. of water, and 15 c. c. of alcohol. Then the apparatus was carefully made tight, 15 c. c. of saturated sodium carbonate added by means of the separatory funnel, and the water pump turned on. The temperature of the water bath was then raised to 25° to 29° C., at which temperature it was held for 3 hours and then raised to 40° to 43° C. The water pump reduced the pressure to 21 to 52 mm. mercury, the average being 28 mm.; 50 c. c. portion of a 4 per cent boric acid solution were used in the Peligot tubes to receive the ammonia. The Peligot tubes were placed in the same ice bath and attached by a Y to the same water pump. Near the end of the 6 to 7 hour distillation period 15 c. c. more of alcohol were cautiously added through the separatory funnels. The distillation was continued 20 to 30 minutes longer, during which time the particles of ammonia-containing moisture that collected in the necks of the flasks and the conducting tubes were removed by squirting a stream of boiling hot water against the exterior of the glass. The contents of the Peligot tubes were transferred to Erlenmeyers and titrated against standard sulphuric acid, using brom-phenol blue as an indicator.

Only qualitative tests were made for the nitrogen of nitrites. Portions of the extract used in the nitric nitrogen determination were made neutral and to them were added 1 c. c. quantities of sulphanilic acid mixture (*15*). A red color in the extract from the diseased leaves indicated the presence of nitrites. A faint pink appeared in the extract from the healthy material indicating a trace of nitrite nitrogen.

Total hydrolyzable nitrogen and nitrogen distribution in the hydrolyzed portions were determined as follows: Eight-gram samples with 400 c. c. of 20 per cent hydrochloric acid were boiled for 9 hours under a reflux condenser. The contents of the flasks were then filtered and washed with ammonia-free water until free from chlorine. The filtrate and washings of each 8 gm. sample were thereupon made to 2 liters. Total nitrogen was determined on 500 c. c. aliquots by the Kjeldahl-Gunning method. Other 500 c. c. portions were evap-

orated to dryness on the water bath and the acid amide, humin, and diamino acid nitrogen estimated according to Hausmann's nitrogen distribution method.

Protein nitrogen was determined by Stutzer's method (*21, p. 105; 22, p. 475*). In this method, 1 gm. samples of the dry celery powder were treated in the beaker with water (100 c. c.) heated to boiling and kept on the water bath for from 10 to 15 minutes. Then 2 c. c. of concentrated potassium-alum solution and 15 c. c. (0.45 gm. $Cu(OH)_2$) of Stutzer's solution [3] were added and the mixture stirred thoroughly. On cooling, it was filtered and thoroughly washed with water. The residue and filter paper was transferred to a Kjeldahl flask and the "protein nitrogen" oxidized by the Kjeldahl-Gunning method.

The nitrogen in the filtrate and washings from the $Cu(OH)_2$ residue was determined by the Kjeldahl-Gunning method and called nonprotein nitrogen. The results for the analysis are nearly the same as those arrived at by subtracting the figures for protein "N" from those for total "N"; they are at least within the error due to sampling which must be considered in all the estimations.

The results of the analyses by the above-described methods are given in Tables I and II.

TABLE I.—*Average* [1] *nitrogen distribution in healthy celery leaves and in leaves affected with "early blight" (Cercospora apii)*

[Total nitrogen expressed in per cent of oven-dried leaves: Healthy, 4.77 per cent; blighted, 2.94 per cent. Nitrogen distribution expressed in per cent of total nitrogen]

Form of nitrogen	Blighted	Healthy
Nitric nitrogen:		
Scales's Zn-Cu couple	5.3	8.7
Schulze-Tieman	4.0	6.7
Ammonia nitrogen	2.6	1.3
Nitrogen of nitrites	(2)	(3)
Total hydrolyzable nitrogen	88.3	90.9
Acid amide nitrogen	16.5	16.7
"Humin" nitrogen	10.6	6.5
Diamino-nitrogen	15.5	16.8
Monoamino nitrogen	45.6	50.7
Protein nitrogen	74.4	68.3
Nonprotein nitrogen	25.3	29.7

[1] For data from which these averages are computed see Table II.
[2] Strongly positive test.
[3] Faint trace.

METHODS USED WITH CELERY LEAVES ATTACKED BY SEPTORIA APII

Mature celery leaves of approximately the same age, taken from plants of the Easy Bleaching variety grown on muck soil at Kalamazoo, Mich., were used. The diseased leaflets used for analysis had spots occupying from one-tenth to one-sixth of the leaf area, while those called healthy were apparently free from spot. The samples were dried for two days on cheesecloth over a steam radiator (temperature 40° to 50° C.) and then were pulverized and dried further in an electric oven held at 50° C. Samples were then sealed in jars.

[3] Preparation of Stutzer's reagent: 100 gm. of $CuSO_4+5H_2O$ were dissolved in 5 liters of water to which 2.5 c. c. of glycerol were added and then enough NaOH to render the solution faintly alkaline. The precipitate was filtered and washed with water containing 5 c. c. glycerol per liter until free from alkali. The precipitate was then taken up with water containing 10 per cent glycerol and the amount of $Cu(OH)_2$ per c. c. determined by the following method: 2 c. c. of Stutzer's solution were treated with 2.5 c. c. of concentrated HNO_3 and 25 c. c. of water and then boiled. Treated with 5 c. c. of bromine water, boiled, treated with NH_4OH, boiled, treated with acetic acid, boiled, cooled, 3 gm. KI added, and titrated with $Na_2S_2O_3$.

TABLE II.—*Analyses of celery leaves—Healthy leaves contrasted with leaves affected with "early blight" (Cercospora apii)*

[Data expressed in percentage of the oven-dried celery leaves]

Determinations	Blighted			Average	Healthy			Average
Moisture content:								
Field material	79.4	76.3		77.8	80.0	80.16		80.08
Greenhouse	75.1	73.8	77.3		84.15	84.25	83.15	
	74.2	77.75	77.0	75.86	84.7	84.1	84.4	84.125
Total nitrogen, Kjeldahl-Gunning method (leaves ground)	2.93	2.92	2.98	2.943	4.78	4.71	4.83	4.773
Nitric nitrogen:								
Scales's Zn-Cu couple method	.156	.156	.1601	.1574	.421	.417	.421	.4197
Schulze-Tieman, no-gas method	.118	.121	.1195		.3255	.320	.325	.323
			.118	.1191				
Ammonia nitrogen, Grafe's method	.0783	.081	.0769	.07693	.06045	.0632	.0714	.06456
			.07145				.0632	

Nitrogen of nitrites, qualitative tests	Present-good test			Average	Faint trace			Average
Total hydrolyzable "N," Kjeldahl-Gunning		2.601				4.342		
Nitrogen distribution, Hausmann's method:								
Acid amide nitrogen	0.4843	0.4911		0.4877575	0.7899	.80725		0.7986
"Humin" nitrogen	.3160	.3091		.31259	.3229	.30913		.3160
Diamino acid nitrogen	.4602	.4533		.456825	.8107	.797		.8038
Monoamino acid nitrogen	1.340	1.347		1.3443	2.418	2.428		2.4233
Protein nitrogen, Ritthausen-Stutzer method	2.174	2.215	2.188	2.1923	3.261	3.281	3.268	3.27
Nonprotein nitrogen "N," in filtrate after precipitation with Stutzer's reagent $CuOH_2$	.759	.7383	.7383	.7452	1.422	1.415	1.415	1.4173

Analyses of the *Septoria apii* material and the corresponding healthy leaves were made by different methods, for the most part, than those used in the spinach investigations. These methods gave results that were very satisfactory and for convenience of manipulation seem to be desirable. Brief directions for carrying out the various tests are included, but for complete instructions the original articles should be consulted.

METHODS OF ANALYSIS USED ON MATERIAL AFFECTED WITH SEPTORIA APII

In the determination of the total nitrogen the method of Davisson and Parsons (7) was followed. One-gram samples of the material were placed in 500 c. c. Kjeldahl flasks, 100 c. c. of distilled water added, and the suspension shaken for a short period (2 to 5 minutes). Two drops of kerosene, 1 gm. of Devarda's alloy, and 1 c. c. of saturated sodium hydroxide solution were then added; the dissolved nitrates and nitrites reduced to ammonia were caught in the tower containing sulphuric acid (27 c. c. concentrated acid plus 8 c. c. water). The complete reduction of the nitrates was assured by heating the solution to boiling in minimum time and then heating

over a low flame for 20 minutes. At the end of that period the material was again brought to vigorous boiling and the flame removed, the sulphuric acid containing the reduced nitrates being drawn back into the Kjeldahl flask. Twenty-five to 30 c. c. of distilled water were then poured upon the glass beads in the tower and the solution in the flask again brought to boiling; then the flame was removed and the wash water drawn into the flask. This washing was repeated four times, thus assuring complete transference of the sulphuric acid to the digestion flask. The material was then digested according to the Kjeldahl-Gunning method, the potassium sulphate not being added until all the water was boiled off. The digested material was diluted to 175 c. c., 25 c. c. of K_2S solution (40 gm. K_2S per liter) added, the whole made strongly alkaline with saturated NaOH and distilled as in the ordinary Kjeldahl procedure.

For the determination of nitric nitrogen Strowd's method (*20*) was employed. Eight grams of the material were extracted by shaking vigorously for 5 minutes with 250 c. c. of cold distilled water. The mixture was allowed to stand for 20 minutes and again shaken for 5 minutes. The extract was filtered and determinations made on aliquot portions. The portions were diluted to 250 c. c. in 500 c. c. Kjeldahl flasks and 2 drops of kerosene and 5 c. c. of saturated NaOH added to each. To half of the samples 1 gm. of Devarda's alloy was also added. All samples were connected immediately to a Kjeldahl distillation apparatus, heated over a very low flame for 1 hour and then 150 c. c. distilled over and caught in 50 c. c. of 4 per cent boric acid solution. Care was taken that the controls containing no alloy were distilled at the same rate as those solutions containing the reducing metal. The distillate was titrated against standard acid, using brom-phenol blue as an indicator, as in the Scales method. The difference between the readings of the control samples and those reduced by the alloy is due to the nitrate and nitrite nitrogen.

The determination of ammonia nitrogen was made by the aeration method of Folin (*10, p. 499–500*). One-gram samples of the healthy and diseased materials were placed in the aeration tubes and to each were added 10 c. c. of water, 1 c. c. of saturated potassium oxalate and 0.5 gm. of anhydrous sodium carbonate. The aeration was effected by means of a water pump, the rate being determined by the tendency of the suspensions to foam over. Aeration was continued for 12 hours. The ammonia was caught in 4 per cent boric acid and titrated as described.

Only a qualitative test for nitrite nitrogen was made. Portions of the extract used in nitric nitrogen determinations as in Jodidi's procedure for spinach were made neutral and to them was added 1 c. c. quantities of the sulphanilic acid mixture. No nitrite was found by this method.

A different method of extraction than that used by Jodidi's procedure was then employed for the test. Ten grams of celery were extracted for 5 minutes with 150 c. c. of distilled water and the infusion thus obtained was filtered through fine Swedish filter paper. The filtrate which had a brown color was partially clarified by shaking for 2 minutes with 10 gms. of Lloyd's reagent with subsequent filtering; 25 c. c. of this filtrate was treated with 3 gms. of Lloyd's

reagent and filtered again; 15 c. c. of this last filtrate was diluted to 50 c. c. in a Nessler tube and tested for nitrite with the sulphanilic acid mixture (*2, p. 23*). The extract from diseased material gave a very strong pink, showing presence of nitrites, whereas a similar procedure with healthy material and with blanks gave no reaction.

To determine total hydrolyzable nitrogen, samples of 4 gms. each of the dried powder in 200 c. c. of 20 per cent hydrochloric acid were boiled for 9 hours in 500 c. c. Kjeldahl flasks under a reflux condenser. The "humin" nitrogen was then filtered off and washed. The filtrate and washings were then made up to liter. Total hydrolyzable nitrogen was determined by Kjeldahlizing 250 c. c. portions of the filtrate.

Nitrogen Distribution in the Hydrolyzed Material

The filter paper bearing the "humin" nitrogen precipitate was returned to the Kjeldahl flasks used in refluxing, and the digestion and distillation carried out by the Kjeldahl-Gunning method. The nitrogen recovered was called "humin."

Acid-amide nitrogen was determined by using aliquot portions of the hydrolyzed material which were made slightly alkaline with strong alkali, and the acid-amide nitrogen forming ammonia, together with the ammonia nitrogen present, was aerated off as in the ammonia nitrogen determinations.

Total amino nitrogen was determined by use of the Van Slyke micromethod. To avoid the interference of the presence of ammonia and amido nitrogen, the aerated solutions from the acid-amide nitrogen determinations after neutralization with sulphuric acid were used.

The protein nitrogen was determined by the method of Stutzer in which 1 gm. samples of the dry celery powder were treated as described in the work with the Cercospora material.

The figures for nonprotein nitrogen were obtained by computation.

The results of the analyses by the above described methods are given in Tables III and IV.

Table III.—*Average nitrogen distribution in healthy celery leaves and in leaves affected with Septoria leaf spot (Septoria apii)* [1]

[Total nitrogen expressed in percentage of oven-dried leaves: Healthy, 5.10 per cent; diseased, 4.38 per cent. Nitrogen distribution expressed in terms of total nitrogen]

	Diseased	Healthy
Nitric nitrogen	5.7	5.8
Ammonia nitrogen	1.3	0.3
Nitrogen of nitrites	[2]	[2]
Total hydrolyzable nitrogen	84.9	88.8
Acid amide	11.8	13.5
"Humin"	10.9	9.6
Total amino nitrogen	43.5	47.1
Protein nitrogen	72.6	67.8
Nonprotein nitrogen	27.1	32.1

[1] For data from which these averages are computed see Table IV. [2] Present. [3] Absent.

TABLE IV.—*Analyses of celery leaves.—Healthy leaves contrasted with leaves affected by Septoria leaf spot (Septoria apii)*

[Data expressed in percentage of oven-dried celery leaves]

Blighted leaves				Average	Healthy leaves			Average
Total nitrogen	.....	4.378	4.374	4.376	5.037	5.15	5.12	5.102
Nitric nitrogen	.....	.2501	.2501	.2501	.285	.310	.....	.297
Ammonia nitrogen	.0621	.0552	.0621	.0598	.0207	.0138	.0138	.0161

Nitrite nitrogen	Positive test			Average	Negative test			Average
Total hydrolyzable nitrogen	.....	3.705	3.726	3.715	4.485	4.581	.....	4.533
Distribution of above:								
Acid amide[1]	.5175	.5175	.5175	.5175	.69	.69	.....	.69
"Humin"	.479	.....	.....	.479	.4623	.....	.....	.4623
Total Amino	1.897	1.911	.....	1.904	2.423	2.39	.....	2.406
Protein nitrogen	3.14	3.22	3.20	3.18	3.46	3.45	3.47	3.46
Nonprotein nitrogen	1.236	1.156	1.176	1.189	1.642	1.652	1.632	1.642

[1] Ammonia nitrogen not subtracted.

DISCUSSION

A comparison of the figures for the two leaf diseases of celery shows that there is in the case of each disease a lower total nitrogen content in the diseased than in the healthy leaves. This is the striking thing in the analysis. Whereas the difference between healthy and diseased leaves is somewhat greater with the *Cercospora apii* material than with *Septoria apii*, this variance is not believed to be significant since the extent of the diseased tissue was greater with the *Cercospora apii* material than with that from *Septoria apii*. The consistently lower total nitrogen content is the important consideration.

There is a lower nitric nitrogen content in diseased leaves. The difference is not significant in the case of *Septoria apii* material, but is pronounced in the case of *Cercospora apii*.

Ammonia nitrogen, in the case of both the *Cercospora apii* and the *Septoria apii* tests, was greater in the diseased material than in the corresponding healthy material. While the differences between healthy and diseased material is less in the case of *Septoria apii* than with *Cercospora apii*, this is not to be taken as a characteristic difference always to be expected. It is rather to be referred to the percentage of diseased material present in the samples. It has commonly been noticed that with leaves extensively affected with *Septoria apii* an ammonia odor is very noticeable.

With *Cercospora apii* material, nitrite was definitely shown to be present by using Jodidi's technique. It was only faintly evident in the healthy, control material. Nitrite could not be so demonstrated with *Septoria apii* material, although tested repeatedly. By a different method of extraction, however, abundant evidence of presence of nitrites was obtained.

With *Cercospora apii* material, the diamino acid content was determined, the monoamino nitrogen being obtained by difference. The healthy leaflets showed the greater per cent of these compounds. With *Septoria apii*, total aminoacid nitrogen was determined, and here also the amount in the healthy leaflets exceeded that of the

diseased leaflets. With diseased leaves, the protein nitrogen was greater than that present in the corresponding healthy control leaflets, in the case of both fungi. The percentages of humin nitrogen was noticeably greater in the diseased leaves.

This outline of the relative nitrogen constituents for healthy and diseased celery shows that with these two parasitic diseases the results of analyses follow closely those of spinach mosaic and those of the unknown cabbage disease to which reference has been made.

TABLE V.—*Deviation of diseased material from healthy material—Comparison of celery diseases with results obtained by Jodidi et al. with spinach and cabbage*

[Hot water extract of spinach and cabbage was used for the acid amide, "humin," diamino, and monoamino tests. The results are expressed in percentage of total nitrogen]

	Celery		Spinach mosaic	Cabbage unknown disease [1]
	Cercospora apii	Septoria apii		
Nitric nitrogen	−3.4 (−2.7)	−0.1	[2] −1.22	−6.42
Ammonia nitrogen	+1.3	+1.0	[2] +0.63	
Nitrite	(+)	(+)	[2] (+)	(+)
Total hydrolyzable nitrogen	−2.6	−3.9		
Acid amide	−0.2	[4] −1.7	[2] −2.89	[4] −7.19
"Humin"	+4.1	+1.3	[2] +0.94	+0.81
Diamino	−1.3	−3.6	[2] −2.33	−4.04
Monoamino	−5.1			
Protein nitrogen	+6.1	+4.8	[3] +9.29	+21.72
Relation of total nitrogen of diseased to healthy material	61	83	[3] 77	77

[1] Figures computed from Tables II, III, and IV (leaves), Jodidi (*12*).
[2] Figures computed from sample A, Table V, p. 1891, Jodidi (*12*).
[3] Figures computed from Tables III and IV, Jodidi et al. (*13*).
[4] Plus ammonia nitrogen.

It will be noted that in every case the deviation of diseased material from healthy is of the same character. Jodidi, because of the similarity of the chemical picture of diseased cabbage and spinach, deduced that the cabbage disease was of mosaic nature. The results of the analyses here presented show that such a deduction is unwarranted.

The results here obtained seem best interpreted from the point of view of metabolism of fungi. A parasitic fungus maintains itself by establishing a food and water relation with a host. From the nitrogenous material of the host it builds its own kinds of proteins. Since Nägeli's classic researches (*18*), it has commonly been accepted that the splitting off of ammonia is the course of protein decomposition by microorganisms. The presence of ammonia in cultures of fungi on richly nitrogenous media has often been demonstrated. Wehmer (*26*) in cultures of *Aspergillus niger* on peptone solution obtained marked formation of ammonia. Further evidence of the formation of ammonia from proteins have been given by many others with a wide range of organisms (*4, p. 310*). But there are intermediate steps in the process and these are poorly known. The work of Butkewitsch (*6*) may be cited as throwing some light on this process. *Apergillus niger*, *Penicillium glaucum*, *Mucor racemosus*, and *Rhizopus nigricans* were shown by him to split off not only ammonia from peptone and fibrin, but also aminoacids, leucin, and tyrosin. (See also Klotz, *14*.) These products were in turn decomposed. From the work of various investigators it has been shown that the process is

enzymic and in various organisms, proteases of various types and enzymes which split amide bodies have been demonstrated. (Waksnan, *24*, *25*.)

In the metabolism of fungi and bacteria the reduction of nitrates to nitrites and to ammonia is of common occurrence (*11*).

The loss of nitrogen in the celery leaves, through the agency of the two fungi, is believed to be not essentially different from the loss of nitrogen from a medium in which the organisms are growing. In short, the fungus uses the nitrogen compounds of the leaf tissue for food. Behrens (*3*) quotes the work of Müller-Thurgau who had noted the lower nitrogen content of the juice from rotted grapes as compared with juice from sound grapes. In another series of tests Müller-Thurgau grew pure cultures of Botrytis upon the grape must and found that the nitrogen content became only one-fifth of the initial amount after 21 days of culturing.

It would seem that the effect of the parasites in the celery disease is to bring about a loss of total nitrogen. The mechanics of the process in cases of parasitism is not known and the use of the term "denitrification" in this connection is misleading. Because of the presence of a trace of nitrite in diseased tissue Jodidi (*12*) has suggested that the action of this compound upon the aminoacid group liberated free nitrogen, by a reaction practically similar to the Van Slyke reaction. He presents no evidence to support this suggestion.

The loss of nitrogen as commonly brought about by organisms seems to be by the splitting off from proteins ammonia which is later liberated. Reasoning from studies on nitrogen metabolism of fungi in cultures the following processes may be assumed to take place (Klotz, *14*): As the strictly carbonaceous food components, such as the carbohydrates, of a lesion become exhausted, probably the nitrogen compounds, as the amino acids, peptones, and proteins must be decomposed to furnish the parasite with carbon requisite for metabolism, especially respiration, or the organism may draw upon its own cell reserves for the nitrogen and carbon to continue life processes. Where the proportion of the nitrogen to the nonnitrogenous complex of either the substrate or of the mycelium itself is greater than is required in the metabolic processes, in protein synthesis (or protoplasmic repair) the excess nitrogen appears as NH_3 and is lost as such. The basis for the above suggestions is to be found in the behavior of fungi in culture since it was found that ammonia was never formed in or excreted into the culture fluid when sugar was present. The disappearance of carbohydrate from the culture medium was found to be synchronous with the appearance of ammonia and with the beginning of autolysis of the fungus. Ammonia was found to be the chief nitrogenous product of the splitting of peptone of the medium in the absence of another carbon source, but in the presence of dextrose NH_3 was reassimilated.

Loss of total nitrogen in the later stage of necrotic diseases is therefore to be expected if the relation to the host is comparable to the relation of a fungus to a culture medium.

Assuming that this is the case in typical parasitic diseases, is one justified in concluding that whenever there is a condition where lower total nitrogen occurs that one is confronted with the work of parasites?

The similarity of the chemical picture of disease in the spinach and in the celery points very strongly to a parasitic agency being at work in the spinach. But it must be borne in mind that it is not known at all what effects these various derangements of normal nutrition produce on plant growth, and exactly as some of these conditions produce spots, lesions, overgrowths, etc., which simulate the diseases brought about by parasites, so a disturbed physiology from other than parasitic agency may give a chemical aspect which simulates more or less closely the condition which accompanies these two diseases caused by parasitic fungi. It would seem that the effects which environmental conditions produce would be largely of a repressive nature, depressing, for example, the total nitrogen production, and such a condition might also be expected with hypoplastic diseases. Necrotic diseases of the types studied, on the other hand, are destructive, reducing that which has already been produced.

With such a criterion, the analyses given when compared with those of spinach go to emphasize that in the case of an infectious disease, such as spinach mosaic is known to be, one may be dealing with a parasite whose effect upon the host is not dissimilar to known parasites, but the similarity of chemical relations alone does not permit judgment in this matter. As far as the cabbage disease is concerned, its infectious nature has not been shown and the evidence at hand does not warrant judgment on purely chemical grounds, as to its parasitic nature. Surely the similarity of chemical analyses is not proof that the cabbage disease is a mosaic disease.

The interpretation of disease production on the basis of the parasite simply establishing a food and water relation with the host opens the question whether disease production by parasites in general is to be interpreted on such simple grounds. Is the living together of host and invader in all types of parasitism, especially the higher types, merely a food and water relation and is the matter of specialization of a parasite upon a species or upon related species determined by the ability of the parasite to invade, grow, and to wrest food from the reserve stuffs of the host?

The data here presented deal with organisms of not a very high specialization in parasitism. These organisms simply produce a necrotic condition in the host. There is no stimulation of the host nor any evidence of tolerance by the tissues invaded. Whether toxic substances are produced by the invaders is not known. These two organisms, though readily grown in pure cultures on ordinary laboratory media of complex nature or on synthetic media of simple salts and sugar, have not been found to be omnivorous in their parasitic habit. Both, on the contrary, are extremely selective in their parasitism, attacking only the celerylike plants (*Apium graveolens*).

The analyses seem to show that the striking thing in the disease production is the mere feeding on the host tissue, the disintegrating of protein in the processes of metabolism. It would seem plausible to suggest that with the necrosis-producing parasites at least, the ability to appropriate food is a very important factor in parasitism. The fungus must first be able to invade and tolerate the conditions within the host, not being walled off or killed by acid or some cellular product. After these barriers are passed, the degree of disease production may largely be correlated with the ability to attack the

food substance of the host, especially the proteins. As in higher plants and animals, the breaking down of proteins and other complex nitrogenous compounds by fungi is accomplished by proteolytic enzymes. These may arise from the host cells themselves in the form of autolytic enzymes which may be released for action by the conditions which the parasite sets up, or the invader may furnish the proteolytic enzymes. By a study of these conditions and especially by studies on the fungous proteases one shall be approaching closely toward a solution of the problem of selective parasitism. Here also lies the domain of immunity to disease, and biochemical studies to determine the nature of immunity must logically turn in this direction.

SUMMARY

Comparative analyses of healthy and diseased celery leaves affected with *Cercospora apii* and *Septoria apii* have been made and it has been found that in the leaves affected with these necrotic diseases there is a lower percentage of total nitrogen in the diseased than in the healthy tissue. Nitrites are present in the diseased material. A comparison of the nitrogenous compounds present show in per cent of total nitrogen, greater ammonia, greater humin, greater protein, less hydrolyzable, less acid amide, less basic and less nonprotein nitrogen in the diseased than in the healthy tissues.

It is believed that these results can best be explained as due to the decomposition of the host by the parasite in a simple food relation.

The existence of a similar chemical picture in the spinach mosaic and in the unknown cabbage disease studied by Jodidi and his associates does not warrant the assumption that the spinach mosaic is of parasitic nature, although the results are very suggestive, nor can the cabbage disease be diagnosed on the basis of mere chemical analyses as a true mosaic.

The importance of the nitrogen metabolism of parasitic fungi is stressed as a possible explanation of selective parasitism and as a point of attack in immunological research.

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